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Sex Hormones, Type 2 Diabetes Mellitus, and Chronic Kidney Disease – An Epidemiological Perspective

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Summary

Sexual dimorphisms observed for type 2 diabetes (T2D) and chronic kidney disease (CKD) suggests involvement of sex hormones in disease development. While higher androgen levels are metabolically beneficial for men, hyperandrogenism is metabolically detrimental for women. However, associations between female sex hormones such as estrogens and progestogens, and T2D remain understudied. Further, potential associations between sex hormones and CKD have not been extensively studied to-date. Among patients with kidney failure, men with low testosterone (T) levels have a higher risk of premature mortality while it is unclear whether similar associations exist for estradiol (E2) among women. Of note, potential associations with incident cardiovascular disease (CVD) and health-related quality of life (HRQOL) have not been reported among patients with kidney failure.

Data from the German Cooperative Health Research in the Region of Augsburg (KORA) study was used to examine the putative role of endogenous sex hormones in T2D and CKD development. Meanwhile, data from the Canadian Kidney Disease Cohort Study (CKDCS) were used to determine the associations between endogenous sex hormones, premature mortality, and health outcomes in hemodialysis (HD) patients. Specific research questions have been addressed in four papers comprising the foundation of this doctoral thesis.

Paper 1 focussed on female sex hormones (i.e. E2, progesterone (P4), and 17α-hydroxyprogesterone (17-OHP)), sex-hormone binding globulin (SHBG), and their associations with insulin- and glucose-related traits, as well as glycemic deterioration. In men, higher E2, P4, and 17-OHP levels were cross-sectionally associated with lower fasting insulin and higher insulin sensitivity. In women, higher E2 and P4 levels were cross-sectionally associated with higher fasting glucose and HbA_{1c}. While no prospective associations were noted among men, postmenopausal women with higher 17-OHP levels were more likely to experience glycemic deterioration.

In Paper 2, we investigated the associations of androgens [i.e. T and dihydrotestosterone (DHT)] and SHBG with kidney function and CKD. We specifically assessed whether non-linear associations exist, and whether T2D could modify any putative associations. In men, our results suggest a U-shaped

association between SHBG and follow-up eGFR. In women, a reverse J-shaped association was seen between DHT and incident CKD. Furthermore, T2D modified some associations.

Papers 3 and 4 focussed on hemodialysis (HD) patients. We assessed the prospective associations of T (Paper 3) and E2 (Paper 4) with mortality, incident CVD and change in health-related quality of life (HRQOL) measures in men and women, respectively. In men, no associations were observed between T and mortality as well as incident CVD. However, men with low T had lower Health Utility Index Mark 3 (HUI3) scores and Kidney Disease Quality of Life Physical Component Scores (KDQOL12-PCS). In women, no linear associations were observed between E2, all-cause mortality, CVD mortality, as well as incident CVD. Splines analyses suggested curvilinear associations between E2 and all-cause mortality, possibly also between E2 and CVD mortality. However, quadratic terms of E2 were significant only for all-cause mortality. Compared to E2 levels in the lowest tertile, E2 levels in the highest tertile were significantly associated with higher all-cause mortality. Further, the association between E2 and HUI3 scores was modified by age: with every 1 SD increase in E2, younger women (<63 years) had better HUI3 scores while older women (≥63 years) had lower HUI3 scores.

Results from epidemiological studies contained herein depicted some (mainly cross-sectional) associations between sex hormones, insulin- and glucose-traits, as well as kidney function in the general population. In patients on HD, some associations were observed between sex hormones and mortality. Moreover, some suggestive links were reported between sex hormones and HRQOL measures. Overall, this thesis contains insufficient evidence to ascertain causality and the relationships observed herein could be bidirectional in nature. Thus, before intervention trials are initiated, further robust evidence is needed from Mendelian Randomisation (MR) studies or larger prospective cohort studies.

Papers included in this thesis

This cumulative thesis is based on the papers as listed below:

Paper 1

Lau LHY, Nano J, Cecil A, Schederecker F, Rathmann W, Prehn C, Zeller T, Lechner A, Adamski J, Peters A, Thorand B. Cross-sectional and prospective relationships of endogenous progestogens and estrogens with glucose metabolism in men and women: a KORA F4/FF4 Study. BMJ Open Diabetes Res Care. 2021 Feb;9(1):e001951.

Impact factor (2021): 4.186

Endocrinology & Metabolism Journal Ranking: 70/146

Paper 2

Lau LHY, Nano J, Prehn C, Cecil A, Rathmann W, Zeller T, Lechner A, Adamski J, Peters A, Thorand B. Associations of endogenous androgens and sex hormone-binding globulin with kidney function and chronic kidney disease. Front. Endocrinol.13:1000650.

Impact factor (2021): 6.055

Endocrinology & Metabolism Journal Ranking: 33/146

Paper 3 (Appendix 1)

Lau L, Wiebe N, Ramesh S, Ahmed S, Klarenbach SW, Carrero JJ, Stenvinkel P, Thorand B, Senior P, Tonelli M, Bello A. Prospective Study of Associations Between Testosterone, Mortality, and Health Outcomes Among Adults Undergoing Hemodialysis. Kidney Int Rep. 2023 Jun 14;8(9):1875-1878.

Impact factor (2022): 6.0

Urology & Nephrology Journal Ranking: 14/124

Paper 4 (Appendix 2)

Lau L, Wiebe N, Ramesh S, et al. Associations of Estradiol With Mortality and Health Outcomes in Patients Undergoing Hemodialysis: A Prospective Cohort Study. Canadian Journal of Kidney Health and Disease. 2023;10. doi:10.1177/20543581231209233

Impact factor (2022): 1.7

Urology & Nephrology Journal Ranking: 82/124

Author contributions

I am the first author and bear responsibility for data integrity and analysis accuracy in all publications herein.

Research questions and study designs for all publications were developed by myself, along with the guidance from my supervisors, Prof. Dr. Barbara Thorand for Papers 1 and 2, and Dr. Aminu Bello and Natasha Wiebe (Research Manager for Canadian Kidney Disease Cohort Study) for Papers 3 and 4. In order to facilitate this, extensive literature reviews and consultations were conducted. This enabled a thorough understanding of sex hormones and their physiological actions specifically within the context of glucose metabolism and the cardio-renal axis. In addition, I developed an understanding of limitations to analyzing data from non-healthy populations (i.e. hemodialysis patients).

During data application stages, I was significantly involved in writing the study proposal and statistical analysis plans for each paper. Furthermore, I was involved in the quality control of sex hormone measurements from the lab of Prof. Jerzy Adamski where I closely worked with Dr. Cornelia Prehn, Dr. Alexander Cecil, and various stakeholders that were involved.

Being a doctoral candidate under the International Helmholtz Research School for Diabetes, I had the opportunity for a research exchange with the Alberta Diabetes Institute, where I was to work with Dr. Aminu Bello. However, due to COVID-19 travel restrictions, my physical presence in Edmonton, Canada could not be realized. Hence, I could not perform the statistical analyses for Papers 3 & 4 due to Canadian Data Protection laws. Accordingly, Natasha Wiebe, performed the statistical analyses on my behalf.

Despite this, I performed the statistical analyses for Papers 1 and 2 using R. All results were interpreted together with my Thesis Advisory Committee and co-authors, which comprised a team of endocrinologists, nephrologists, epidemiologists, and statisticians. I wrote all manuscript drafts, consolidated comments from co-authors, finalized manuscripts based on reviewer comments, and was the liaison between journal editors and co-authors.

Abbreviations

17-OHP	17α-hydroxyprogesterone
BMI	Body mass index
CI	Confidence interval
CKD	Chronic kidney disease
CKDCS	Canadian Kidney Disease Cohort Study
CRP	C-reactive protein
CVD	Cardiovascular diseases
DHT	Dihydrotestosterone
E2	Estradiol
fE2	Free estradiol
fDHT	Free dihydrotestosterone
fT	Free testosterone
eGFR	Estimated glomerular filtration rate
HbA_{1c}	Hemoglobin A1c (glycated hemoglobin)
HD	Hemodialysis
HDL	High density lipoprotein
HPG	Hypothalamic-pituitary-gonadal
HR	Hazard ratio
HRT	Hormonal replacement therapy
HRQOL	Health-related quality of life
HUI3	Health Utilities Index Mark 3
KDQOL	Kidney Disease Quality of Life
KORA	Cooperative Health Research in the Region of Augsburg
MD	Difference in means
MR	Mendelian Randomization
OGTT	Oral glucose tolerance test
OR	Odds ratio
P4	Progesterone
QUICKI	Quantitative Insulin Sensitivity Check Index
SD	Standard deviation
SHBG	Sex hormone-binding globulin
T	Testosterone
T2D	Type 2 diabetes
UACR	Urinary albumin to creatinine ratio

1. Introduction

Diabetes mellitus, characterized by chronic hyperglycemia, is a major public health concern. In 2021, 573 million (~10%) adults worldwide aged between 20 and 79 are living with diabetes [1]. According to the International Diabetes Federation, 6.7 million deaths were ascribed to diabetes [1] – making it one of the leading causes of death worldwide.

Type 2 diabetes (T2D) accounts for 90-95% of all diabetes cases [2]. Progression starts at the onset of prediabetes, where blood glucose levels are elevated but not high enough to be considered T2D yet. Prediabetes is perpetuated by the body's inability to respond appropriately to insulin (insulin resistance) and progressive loss of adequate β-cell insulin secretion. T2D clinically exhibits itself with elevated glucose (fasting and postprandial) and glycated hemoglobin (HbA_{1c}) levels [3].

As T2D is chronic and progressive in nature, complications occur as a result. A common complication is nephropathy: up to 40% of individuals with diabetes develop nephropathy [4] and those with diabetes are more likely to have fast CKD progression (estimated glomerular filtration rate (eGFR) declines >4 ml/min/1.73 m^2 per year) [5]. Current CKD interventions target several pathogenic pathways, but are only able to slow CKD progression [6]. As CKD progresses to kidney failure (defined as eGFR <15 ml/min/1.73m^2), mortality rates and healthcare costs increase. Compared to individuals without diabetes, those with diabetes progress faster to kidney failure.

Many patients with kidney failure undergo hemodialysis (HD). Although dialysis technology and patient access to HD has improved considerably since its inception over six decades ago, mortality and impaired quality of life remains high [7]. Patients with kidney failure have an abnormal sex hormone profile presented by hypothalamic-pituitary-gonadal axis disturbances [8], T deficiency in men [9-11], and low E2 levels [12, 13] in women. Low T and E2 levels are also linked with frailty, muscle wasting, osteoporosis, and poor physical function [14] – all of which can lead to increased mortality and lower quality of life.

Sex differences in T2D and CKD are apparent in adults: men have higher T2D and CKD risk, and progress faster to ESKD compared to women [15-22]. The female sex protects against kidney disease progression among younger women

[23], however, after menopause, this female sex advantage diminishes due to numerous physiological changes during the postmenopausal stage including lower circulating estrogen levels. While sex differences in T2D and CKD disease pathology are multi-faceted [20, 24], this thesis focuses on the role of sex hormones.

1.1 Sex hormones and sex hormone-binding globulin (SHBG)

Sex hormones are primarily secreted within the reproductive organs, and have many physiological roles in the human body. Apart from their main role in modulating the formation of secondary sexual characteristics, sex hormones are also implicated in the function of various organ systems that control metabolic and kidney function. Sex hormones are classified into three distinct classes: androgens, estrogens, and progestogens.

1.1.1 Androgens

Testosterone (T) is the principal androgen in humans. In men, T is produced in the testicles. In women, T is produced across several sites: ovaries, adrenal glands, and peripheral tissues from precursors produced by the ovaries and adrenal glands. 5α-reductase converts T to dihydrotestosterone (DHT) [25]. Both T and DHT bind androgen receptors (ARs). As DHT has higher affinity for ARs and takes longer to dissociate from ARs, DHT is more potent than T [26, 27]. ARs are expressed at multiple sites such as skeletal muscle [28], liver [29], pancreatic β-cells [30], and kidneys [31], among others. Although ARs exist in both men and women, T and DHT can induce sex-specific tissue responses.

It is well-known that T deficiency promotes obesity and insulin resistance in men [32-34]. In line with this, testosterone replacement therapy (TRT) can improve glycemic control, insulin sensitivity, lipid parameters in men with hypogonadism and T2D [35]. While higher T levels are metabolically beneficial for men, androgen excess could promote β-cell malfunction in women [36]. Polycystic ovarian syndrome, the main cause of hyperandrogenism, predisposes women to T2D [37]. Higher T and DHT levels can cause chronic AR activation in β cells. Consequently, insulin hypersecretion [38] and secondary β-cell failure occurs [36].

T can increase kidney blood pressure in men [31] and worsen kidney fibrosis in male mice [39]. In female mice, while T can induce kidney injury [40], antagonizing T can protect against kidney injury [41]. With age, postmenopausal women produce more androgens. As androgens could potentially harm kidney function among women [42], further studies are warranted.

1.1.2 Estrogens

Estradiol (E2) is the principal estrogen. It is mainly produced in the ovaries of premenopausal women. In men, the testicles produce ~20% of circulating E2. The rest is produced locally at sites such as adipose tissue. Here, aromatase converts T to E2 [43]. E2 levels fall with age in men, but more so in women as they become postmenopausal.

There is evidence that E2 protects β-cell survival and controls insulin production in female mice [44-46]. When E2 levels are within the physiological range, E2 acts on skeletal muscle and liver to mediate beneficial metabolic effects, such as improved insulin sensitivity and glucose tolerance, anti-lipogenesis, and fat mass reduction in female mice [47, 48]. In contrast, supraphysiological E2 levels can promote insulin resistance [44].

E2 is renoprotective in animal models. E2 treatment reduced glomerulosclerosis [49], prevented ischemia-reperfusion injury [50], and slows kidney disease progression [51-53].

1.1.3 Progestogens

Progestogens are a group of endogenous or synthetic steroid hormones that bind to progesterone receptors. Progesterone (P4) is the main endogenous progestogen. In women, P4 is primarily produced in the ovaries. During the first nine weeks of pregnancy, the ovarian corpus luteum secretes P4. After this period, P4 secretion shifts to the placenta [54]. In men, P4 is secreted in the testes. Alternate production sites include the adrenal cortex [55]. 17α-hydroxyprogesterone (17-OHP) is the main metabolite of P4 (catalyzed by 17-hydroxylase) [55].

Apart from its role in male and female reproductive health [54, 55], progestogens may be involved in T2D pathophysiology. In animal studies, P4 increased blood

pressure in pregnant mice [56], reduced insulin release [57, 58], and induced β-cell apoptosis [59]. 17-OHP induced hyperglycemia in female mice [60].

The kidney produces P4 and contains progesterone receptors [42, 61]. Despite this, the role of progestogens in kidney physiology and pathology are still unclear. The kidney contains enzymes that metabolize P4 into inactive metabolites [62, 63]. These enzymes also convert pregnenolone (a P4 precursor) to androgen precursors, eventually leading to T and DHT formation [61]. Therefore, T is more likely than progestogens [31] to modulate kidney blood pressure and function.

1.1.4 SHBG

SHBG is produced in the liver and is the main transport protein for sex hormones. SHBG regulates sex hormone bioactivity as bound sex hormones cannot diffuse into target tissues [64]. In men and women, SHBG changes differently with age: in men, SHBG increases linearly with age while in women, SHBG decreases then increases with age [65].

Hormonal and metabolic factors can affect hepatic SHBG production [66-68], and studies have shown that insulin suppresses hepatic SHBG production [66, 69]. Other studies have additionally suggested that high carbohydrate intake and fasting glucose levels, rather than hyperinsulinemia, determine hepatic SHBG production [68, 70]. Epidemiological studies consistently show that SHBG levels are lower among individuals with T2D [71].

In the context of kidney function, there is a current paucity of studies involving SHBG. Results from a rodent study suggest that SHBG promotes androgen uptake into proximal convoluted tubule cells and sustains androgen access to ARs when androgen levels are low [72].

1.2 Sex hormones, T2D, and chronic kidney disease – Epidemiological evidence and gaps

The number of epidemiological studies investigating the associations of sex hormones with T2D, kidney function, and CKD is increasing. Despite this, there are still research gaps that need to be addressed.

1.2.1 Associations between sex hormones, SHBG, and T2D in population-based studies

Numerous observational studies have shown associations between T, E2, and SHBG and T2D. Collectively, in men, lower T, higher E2, and lower SHBG levels are associated with T2D incidence [73] while in women, higher T, higher E2, and lower SHBG levels are associated with T2D development [71, 74-76]. Although epidemiological reports seem to be inconsistent to those of animal studies that describe beneficial effects of E2 on glucose homeostasis, these results are derived from observational studies where causality cannot be determined. Thus, there is insufficient evidence to say that high E2 levels could increase T2D risk. Several reports using Mendelian Randomization (MR) principles (to ascertain causality) suggested that higher T [77, 78] and SHBG levels could lower T2D risk in men and postmenopausal women [78-80].

While T and E2 have been studied extensively in the context of T2D pathophysiology, DHT (a more potent androgen) has not. A study in 852 older men followed over 9.8 years showed that higher DHT levels were associated with lower insulin resistance and T2D incidence [81]. A study in 4516 presumed premenopausal women (aged 31-50 years) followed for 8.1 years did not find an association between DHT and T2D incidence [76]. Notably, T2D studies in premenopausal women are difficult because T2D incidence is low in this population, and can result in low study power.

The role of progestogens in T2D development has also rarely been examined. One study noted associations between higher P4 levels and higher fasting glucose, glycated hemoglobin (HbA_{1c}), and lower HOMA-β (lower β-cell activity) [82] in men and women.

1.2.2 Associations between sex hormones, SHBG, kidney function, and CKD in population-based studies

Compared with diabetes, the role of androgens in CKD pathophysiology has not been extensively assessed. Population-based reports describing relationships between androgens and kidney function are inconsistent. A report described reduced kidney function with lower T levels among men [83], while another one showed no differences [84]. In another study including both men and women, no associations between T and kidney function were apparent in either sex [85].

SHBG is known for its role as a transport protein, but SHBG and its driving factors could also impact CKD development. Higher SHBG levels were associated with better kidney function [85] and could lower CKD risk [86]. However, these associations were seen only among men - indicating a possible sex-specific relationship.

1.2.3 Associations between sex hormones, mortality, and HRQOL in HD patients

T and E2 deficiency are common in men and women with kidney failure, respectively. Sex hormone deficiencies in HD patients are thought to play a role in premature mortality and increased CVD risk.

Men on HD with low T levels have higher mortality risk and worse health-related outcomes as a consequence of lower physical capabilities or anemia [9-11, 87]. Meanwhile, women on HD with either low or high E2 levels have a higher mortality risk [12, 13]. It is still unclear how E2 is prospectively-linked with health-related outcomes.

1.2.4 Knowledge gaps and study limitations addressed by this thesis

Knowledge gaps regarding the role of sex hormones in T2D and CKD development and progression still exist despite an ever-increasing amount of evidence. First, female sex hormones (i.e. estrogens and progestogens) have metabolic actions but few epidemiological studies assessed their potential impact on glucose- and insulin-related traits. Second, putative associations between androgens and kidney function in women have not been sufficiently explored. Third, while some studies assessed associations of T and E2 with mortality in HD patients, these studies are limited by their follow-up time and have not assessed HRQOL.

2. Aim of this thesis

This thesis aims to address current knowledge gaps summarized in section 5.2.4. Figures 1 and 2 illustrate specific research questions that were addressed in four papers forming the basis of the current cumulative thesis.

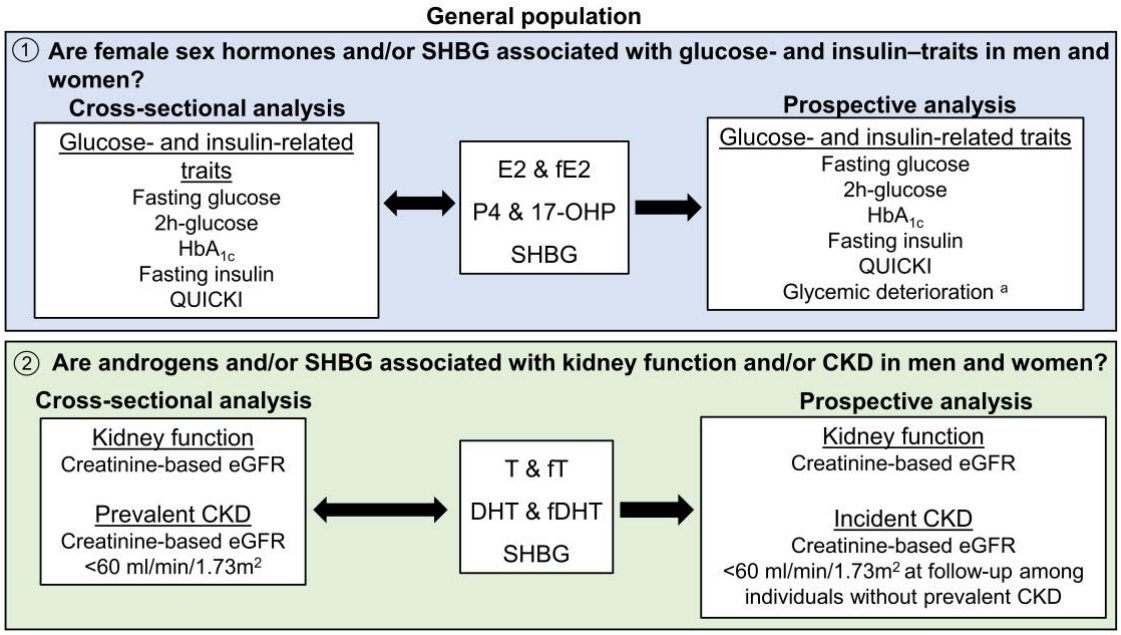

Figure 1. Epidemiological insights into the relationships of sex hormones with glucose- and insulin-related traits, kidney function and the development of T2D and CKD in the general population.

Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CKD: Chronic kidney disease, DHT: Dihydrotestosterone, eGFR: Estimated glomerular filtration rate, E2: Estradiol, fE2: Free estradiol, fT: Free testosterone, P4: Progesterone, SHBG: Sex hormone-binding globulin, T: Testosterone.

[a] Glycemic deterioration was defined as the transition from NGT→pre-diabetes, NGT→T2D, and pre-diabetes→T2D from baseline to follow-up (i.e. F4→FF4)

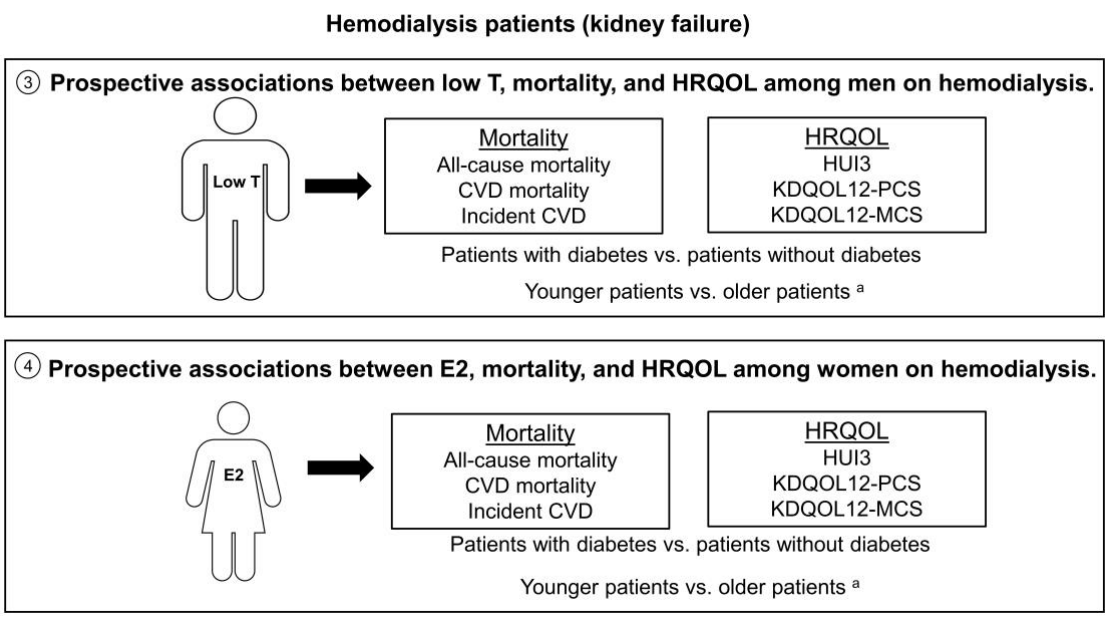

Figure 2. Epidemiological insights into the associations of endogenous T and E2 levels with mortality and HRQOL measures in patients with kidney failure.

Abbreviations: CVD: Cardiovascular diseases, E2: Estradiol, KDQOL12-PCS: Kidney Disease Quality of Life Physical Component Score, KDQOL12-MCS: Kidney Disease Quality of Life Mental Component Score, HRQOL: Health-related quality of life, HUI3: Health Utility Index Mark 3®, T: Testosterone.

[a] Patients were considered younger if below median age (i.e. <63 years) or older if equal or above median age (≥63 years). Median age is 63 years for study populations in Papers 3 and 4.

In Paper 1, we aimed to determine whether female sex hormones and SHBG were associated with impaired glucose metabolism. Specifically, we examined the cross-sectional and prospective associations of female sex hormones and SHBG with glucose- and insulin-related traits, as well as with glycemic deterioration. Furthermore, we examined associations of female sex hormones and SHBG with glycemic deterioration (Figure 1).

In Paper 2, we shifted our focus to male sex hormones (androgens), and specifically assessed the associations of androgens and SHBG with kidney function. Furthermore, we assessed whether these associations differ among individuals with or without diabetes (Figure 2).

While Papers 1 and 2 were based on data from the general population, Papers 3 and 4 focused on patients with kidney failure undergoing HD. In Paper 3, we examined the prospective association of low T with mortality and HRQOL among men with kidney failure. In Paper 4, we assessed prospective linear and non-linear associations of E2 with all-cause mortality and CVD mortality among

women with kidney failure. We further examined prospective associations between E2 levels and HRQOL. In Papers 3 and 4, we assessed whether the presence of diabetes or age would modify these associations.

3. Methods overview

3.1 Study populations and design

For Papers 1 and 2, data from the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 and FF4 studies were used to examine the cross-sectional and prospective associations of sex hormones with impaired glucose metabolism and kidney function in a general population. Pre-menopausal women were excluded from analyses. As for Papers 3 and 4, data from the Canadian Kidney Disease Cohort Study (CKDCS) was used to evaluate the associations of low T and E2 levels with mortality and HRQOL among men and women with kidney failure, respectively.

3.1.1 KORA F4/FF4

The KORA F4 study is a population-based study based in Augsburg, Southern Germany conducted between 2006 and 2008. It involved 3080 adults (32 - 81 years old) out of 4261 individuals who participated in the baseline KORA S4 study in 1999 to 2001. The KORA FF4 study, conducted between 2013 and 2014, was a follow-up examination of the KORA S4/F4 study. 2161 participants that took part in KORA F4 participated in KORA FF4.

In Paper 1, cross-sectional and prospective associations of female sex hormones, and SHBG with glucose- and insulin-related traits, as well as glycemic deterioration were examined. After exclusions, the analysis included 1816 participants (1222 men and 594 women) of KORA F4 (defined as baseline for the present analysis). For the prospective analysis, 1311 participants (921 men and 390 women) with follow-up data at KORA FF4 were included. Participants taking antidiabetic medications were excluded from analyses that were examining continuous glucose and insulin-related traits as outcomes. Information on included covariates is detailed in Paper 1 [88].

In Paper 2, the cross-sectional and prospective associations of androgens and SHBG with kidney function and CKD were assessed. The cross-sectional sample included 1941 participants (1291 men and 650 women), while the prospective sample included 1349 participants (933 men and 416 women). Information on included covariates is detailed in Paper 2 [89].

3.1.2 Canadian Kidney Disease Cohort Study (CKDCS)

Papers 3 and 4 were secondary analyses using data from the CKDCS: a prospective observational study of patients initiating HD across five Canadian centres serving ethnically diverse populations (Vancouver, Calgary, Edmonton, Ottawa, and Toronto). Eligible participants were recruited between February 2005 and February 2011. The CKDCS enrolled all incident adult HD patients aged ≥18 years from the five participating renal programs. Patients that provided informed consent were approached by study staff within eight weeks of commencing dialysis therapy. Data was collected using a structured interview to obtain detailed information on demographics, medical history, social history, and satisfaction with care, supplemented by clinical record linkage. Study visits were conducted at baseline, month 6, annually for five years, then every five years thereafter [90].

3.2 Sex hormone quantification, hypogonadism, and menopausal status

In all study populations, serum sex hormones were measured only at baseline. In Papers 1 and 2, sex hormones were quantified using liquid chromatography-electrospray ionization-tandem mass spectrometry and the Absolute*IDQ* Stero17 Kit (BIOCRATES Life Sciences, Austria) [91]. In Papers 3 and 4, T and E2 were quantified using standardised routine methods, respectively [9, 12]. Mass action equations [92, 93], along with serum albumin and SHBG measurements, were used to calculate fT, fE2, and fDHT.

In KORA F4 (Papers 1 and 2), menopausal status in women was ascertained during an interview by trained medical staff. Women with regular menstrual bleeding were considered pre-menopausal. Women aged <60 years with a hysterectomy (who still had at least one ovary) were considered peri-menopausal. Women who: (1) reported no menstrual bleeding for ≥12 months, (2) reported irregular menstrual bleeding within the last 12 months, or (3) were >75 years of age were considered post-menopausal. Women on HRT and also those who underwent bilateral oophorectomy were excluded.

In Paper 3, T status was grouped using international criteria as follows: low (serum T < 8 nmol/L) and non-low (borderline = 8 - 12 nmol/L and normal >12

nmol/L). fT status was defined as follows: low (fT <180 pmol/L) and non-low (borderline = 180-250 pmol/L and normal <250 pmol/L) [94].

In Paper 4, menopausal status could not be ascertained. Women on hemodialysis experience earlier menopause at around 47 years [95], compared to the general population at around 52 years [96]. Attempts using algorithms to determine menopausal status have been proved unreliable among women with kidney failure [12].

3.3 T2D, glucose- and insulin-related traits, and glycemic deterioration

For Paper 1, in KORA F4 (baseline) and FF4, known-T2D was defined by participants' self-report (validated by a physician or chart review) or current glucose-lowering medication usage. Participants without known T2D received a 75g oral glucose tolerance test (OGTT) [97]. Fasting glucose, 2h-glucose, fasting insulin, HbA_{1c}, and Quantitative Insulin Sensitivity Check Index (QUICKI) were outcomes in Paper 1. QUICKI was used as a measure of insulin sensitivity [98] and was calculated using the formula below:

$$QUICKI = \frac{1}{log_{10} fasting\ glucose\ (mg/dl) + log_{10} fasting\ insulin\ (\mu U/ml)}$$

The outcome glycemic deterioration (Paper 1), was defined as the change from normoglycemia (NGT) to prediabetes, NGT to T2D, and prediabetes to T2D from F4 to FF4. 135 participants with prevalent T2D at F4 were excluded, resulting in a final sample size of 851 non-cases and 278 cases with glycemic deterioration.

In Papers 3 and 4, clinical diabetes diagnoses were determined by reviewing medical charts.

3.4 Kidney function and CKD

In Paper 2, serum creatinine was used to estimate glomerular filtration rates based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula equation [99]. Prevalent CKD was defined as having an eGFR <60

ml/min/1.73m^2 at F4. Meanwhile, incident CKD was defined as having an eGFR <60 ml/min/1.73m^2 at FF4 among participants without prevalent CKD.

3.5 All-cause mortality, CVD mortality, and incident CVD

In Papers 3 and 4, death was determined by chart review. ICD-10 codes were used to define CVD mortality based on an algorithm which was previously published [100]. Information regarding incident CVD events were based on administrative data from Alberta Health (the provincial health ministry). Incident CVD was captured for those without prevalent CVD at baseline and included stroke, transient ischemic attack, coronary artery disease, heart failure, and peripheral vascular disease.

3.6 Health Utilities Index® and KDQOL SF12-P/MCS definitions

Health Utilities Index (HUI) is a conglomerate of generic health profiles and preference-based systems. It serves to measure health status, report HRQOL, and produce utility scores. Currently, the HUI consists of two systems, HUI2 and HUI3, where the latter is used to assess HRQOL in Papers 3 and 4. The HRQOL scoring systems (i.e. HUI3 scores) provide utility scores on a generic scale where dead = 0.00 and perfect health = 1.00.

The Kidney Disease Quality of Life (KDQOL) survey is a kidney-specific measure of the aforementioned HRQOL. The KDQOL-36 is a 36-item HRQOL survey with five subscales, two of which were used as an outcome in Papers 3 and 4; the SF-12 measure of physical (PCS) and mental (MCS) scores. This subscale asks about general health, activity limits, ability to accomplish desired tasks, depression and anxiety, energy level, and social activities. Repeated measurements of HUI3 and KDQOL12-PCS and -MCS scores were available at follow-up timepoints (i.e. baseline, month 6, years 1, 2, 5, and 10).

3.7 Statistical methodology

In Paper 1, multivariable linear regression models were used to estimate the cross-sectional and prospective associations of female sex hormones and SHBG with glucose- and insulin-related traits. To estimate associations of female sex

hormones and SHBG with glycemic deterioration, logistic regression was used. Additionally, non-linear associations were explored using restricted cubic splines. Specific covariates included in regression models have been detailed in Paper 1 [88].

In Paper 2, multivariable linear and logistic regression models were used to evaluate the cross-sectional and prospective associations of androgens and SHBG with eGFR (kidney function) and CKD. Multivariable logistic regression was used instead of time-to-event analysis because exact CKD manifestation timepoints were unavailable. To assess non-linearity, we included a linear and a squared term for androgens and SHBG. Associations that showed significant non-linearity were visualized using restricted cubic splines. All association analyses were adjusted for CKD risk factors [89]. The interaction of androgens and SHBG with baseline diabetes status (i.e. NGT and prediabetes vs. T2D) was evaluated using multiplicative terms. A series of sensitivity analyses were performed: (1) We used a 3-standard deviation (SD) cut-off to exclude participants with extreme androgen levels to determine their impact on estimates. (2) We adjusted for free triiodothyronine (fT3), free thyroxine (fT4), and thyroid stimulating hormone (TSH) to account for its impact on androgen activity. (3) We adjusted for T in SHBG models to determine the independency of SHBG on the outcomes. (4) We excluded peri-menopausal women as sex hormones fluctuate during this phase.

In Papers 3 and 4, Cox proportional hazards models were used to assess associations of T and E2, respectively with all-cause mortality and CVD mortality. In Paper 3, the primary model included low T (i.e. <8 nmol/L) vs. non-low T (≥8 nmol/L). In Paper 4, the primary model was a linear model for a 1-SD E2 increase. Restricted cubic splines with three knots (placed at the 5^{th}, 50^{th}, and 95^{th} percentiles) were used to determine the presence of possible non-linear relationships. Then, quadratic and linear terms for E2 were used for non-linearity testing. Fine and Gray models were used for incident CVD to account for competing risks. Linear mixed effect models were used for repeated outcome measures for HRQOL. Participants were modelled as a random effect and visit number as a fixed effect. Covariates included in multivariable models in Papers 3 and 4 were similar, with small exceptions: SHBG measurements were not available among women (i.e. Paper 4), thus, serum albumin was added to the

models instead. The effect modification by age and diabetes was assessed by including a multiplicative interaction term between sex hormones and age or diabetes into the models.

Different pre-specified sensitivity analyses were performed for each paper. For Paper 3: (1) Serum T was analyzed on a continuous scale. (2) fT measurements were used to reclassify participants into low (<180 pmol/L) and non-low (borderline = 180-250 pmol/L and normal >250 pmol/L) categories using internationally-recognized recommendations [94]. For Paper 4: E2 was investigated as tertiles.

In all papers, men and women were analyzed separately. All sex hormones were z-standardized; therefore, associations were estimated for a 1-SD increase. R (v4.0.5) [101] was used for statistical analyses in Papers 1 and 2. In Papers 3 and 4, STATA/MP 17.0 (www.stata.com) was used. A two-sided *p*-value of <0.05 was used as a significance threshold in all papers.

4. Key findings

Key findings 1 (Paper 1): Among men, female sex hormones were cross-sectionally associated with glucose- and insulin-related traits, but not with glycemic deterioration. Among women, only 17-OHP and fE2 were cross-sectionally associated with selected glucose-related traits. There was some indication that 17-OHP may be related to glycemic deterioration among post-menopausal women.

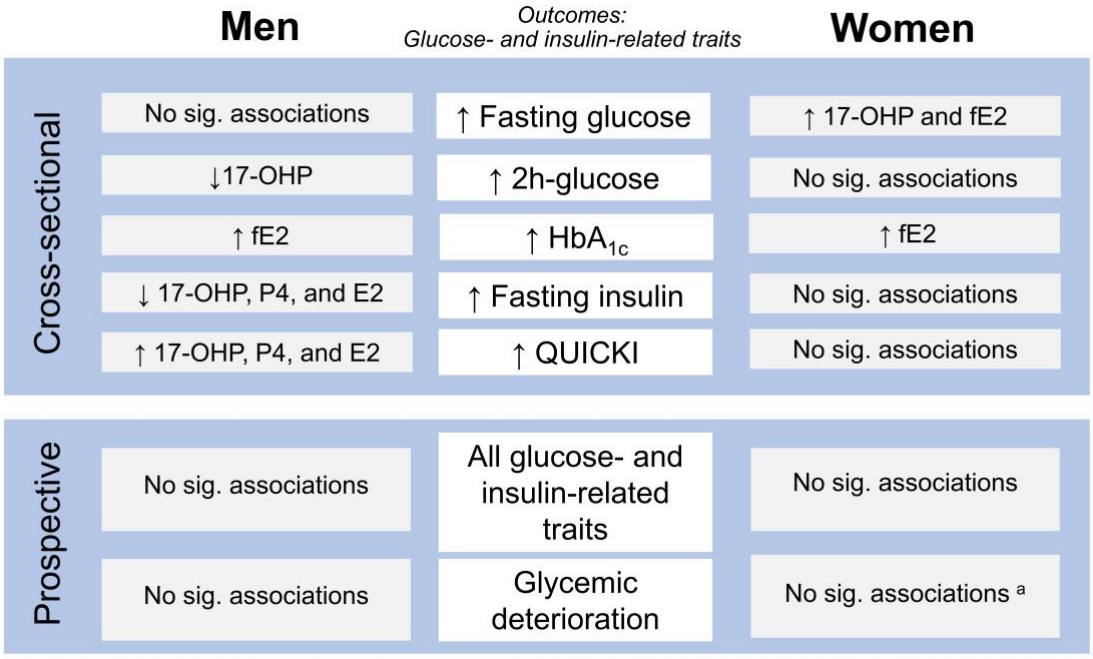

Figure 3. Result summary for Paper 1.

Abbreviations: 17-OHP: 17α-hydroxyprogesterone, fE2: Free estradiol, E2: Estradiol, HbA_{1c}: Glycated hemoglobin, P4: Progesterone, QUICKI: Quantitative Insulin Sensitivity Check Index.

[a] Positive association noted between 17-OHP and glycemic deterioration (OR=1.52, 95% CI 1.03 to 2.26) after perimenopausal women were excluded.

In the cross-sectional analysis of 1222 men in KORA F4, 17-OHP was inversely associated with 2h-glucose [β=−0.067, (−0.120; −0.013)], while fE2 was positively associated with HbA_{1c} [β=0.079, (0.027; 0.132)]. As for insulin traits, 17-OHP, P4, and E2 were inversely associated with fasting insulin [$\beta_{17\text{-}OHP}$=-0.074, (-0.118; -0.030); β_{P4}=-0.047, (-0.088; -0.006); β_{E2}=-0.068, (-0.116; -0.020)] while higher 17-OHP, P4, and E2 levels were associated with higher QUICKI values [$\beta_{17\text{-}OHP}$=0.061, (0.018; 0.105); β_{P4}=0.041, (0.001; 0.082); β_{E2}=0.059,

(0.012; 0.107)]. No significant associations were seen between SHBG and any of the assessed outcomes.

Among 594 women in KORA F4, 17-OHP and fE2 were positively associated with fasting glucose ($\beta_{17\text{-}OHP}$=0.068, (0.014; 0.123); β_{fE2}=0.080, (0.020; 0.141). fE2 was positively associated with HbA_{1c} [β=0.121, (0.062; 0.180)]. No other significant cross-sectional associations were observed among women. After perimenopausal women were excluded, several associations were strengthened: (1) P4 and E2 became significantly associated with fasting glucose [β_{P4}=0.071, (0.014; 0.137); β_{E2}=0.076, (0.014; 0.137)]. (2) P4 became significantly associated with HbA_{1c} [β=0.071, (0.008; 0.133)].

In men and women, no prospective associations were observed of female sex hormones and SHBG with glucose- and insulin-related traits. Initially also no significant associations were seen of female sex hormones and SHBG with glycemic deterioration in men and women. However, after excluding perimenopausal women, baseline 17-OHP levels were positively associated with glycemic deterioration (OR = 1.518, [1.033; 2.264]). There were no significant indications of nonlinearity.

Key findings 2 (Paper 2): Among men, SHBG showed a U-shaped association with follow-up eGFR. Among women, a reverse J-shaped association was seen between DHT and incident CKD while an inverse association was observed between fDHT and incident CKD. T2D was an effect modifier of several associations.

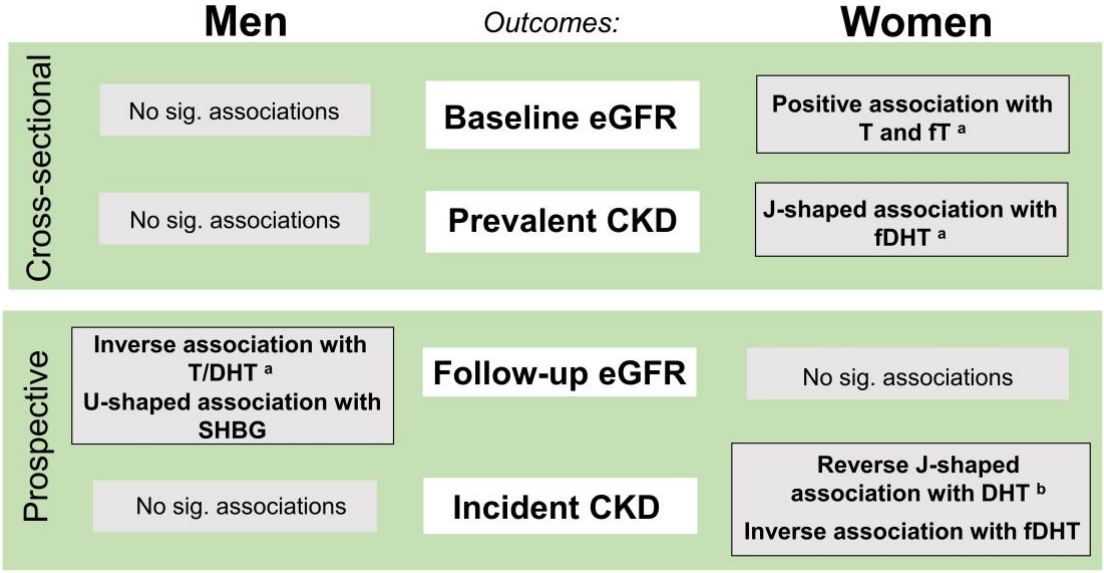

Figure 4. Result summary for Paper 2. Prevalent CKD defined as eGFR <60 ml/min/1.73m^2 at baseline. Incident CKD defined as eGFR <60 ml/min/1.73m^2 at follow-up among participants without prevalent CKD.

Abbreviations: CKD: Chronic Kidney Disease, DHT: Dihydrotestosterone, eGFR: estimated glomerular filtration rate, fDHT: Free dihydrotestosterone, fT: Free testosterone, SHBG: Sex hormone-binding globulin, T: Testosterone.

[a] When extreme androgen values (i.e. values >3SD from the mean) were excluded, in women, the positive association between T, fT, and baseline eGFR became insignificant. In men, the initial prospective association between T/DHT ratio and follow-up eGFR became insignificant.

[b] When thyroid hormones were accounted for, the reverse J-shaped association between DHT and incident CKD did not persist (β_{DHT}=0.588, [0.343; 0.986], β_{DHT^2} = 1.118, [0.868; 1.429]), $P_{non\text{-}linear}$=0.122).

Data from 1293 men (mean age 56 years) and 650 women (mean age 63 years) who participated in KORA F4 were used. Seventy-three men (5.7%) and 54 women (8.4%) had prevalent CKD. In the cross-sectional analyses among men, no associations of androgens and SHBG with baseline eGFR and prevalent CKD

were seen. Sensitivity analysis did not discernably alter these observations. Among women, T and fT were inversely associated with baseline eGFR (β_T=-1.305, [-2.290; -0.320]; β_{fT}=-1.423, [-2.449; -0.397]), but these associations were attenuated to non-significance after excluding women with extreme androgen values. An initial J-shaped association was seen between fDHT and prevalent CKD, but this J-shaped association did not persist. Rather, we saw a significant inverse association (OR_{fDHT}=0.571, [0.328; 0.931]).

Over a median follow-up time of 6.5 years, 81 men (8.8%) and 60 women (15.2%) developed incident CKD. Among men, a higher T/DHT ratio was initially associated with lower follow-up eGFR (β=-0.819, [-1.413; -0.226]) but results did not remain significant after excluding extreme androgen values. The association between SHBG and follow-up eGFR was U-shaped ($P_{non\text{-}linear}$=0.011) and remained significant after accounting for T and thyroid hormones. In women, a reverse J-shaped association was seen between DHT and incident CKD ($P_{non\text{-}linear}$=0.025). This association sustained after excluding extreme values, but not after accounting for thyroid hormones. Also, higher fDHT levels were associated with lower incident CKD risk (OR_{fDHT}=0.613, [0.369; 0.971]). This association remained significant in all sensitivity analyses.

T2D was a significant effect modifier in several instances. Among men with T2D, follow-up eGFR increased with higher T levels ($\beta_{T(Diabetes)}$=1.709, [-0.679; 4.097]). This was in contrast to men without T2D, where follow-up eGFR decreased with higher T levels ($\beta_{T(No\ diabetes)}$=-0.425, [-1.130; 0.279]) ($P_{interaction}$ = 0.041, Figure 5A). Among women, baseline eGFR decreased in general with higher T levels. However, women with T2D had steeper baseline eGFR decline compared to their counterparts without T2D ($P_{interaction}$ = 0.014, Figure 5B).

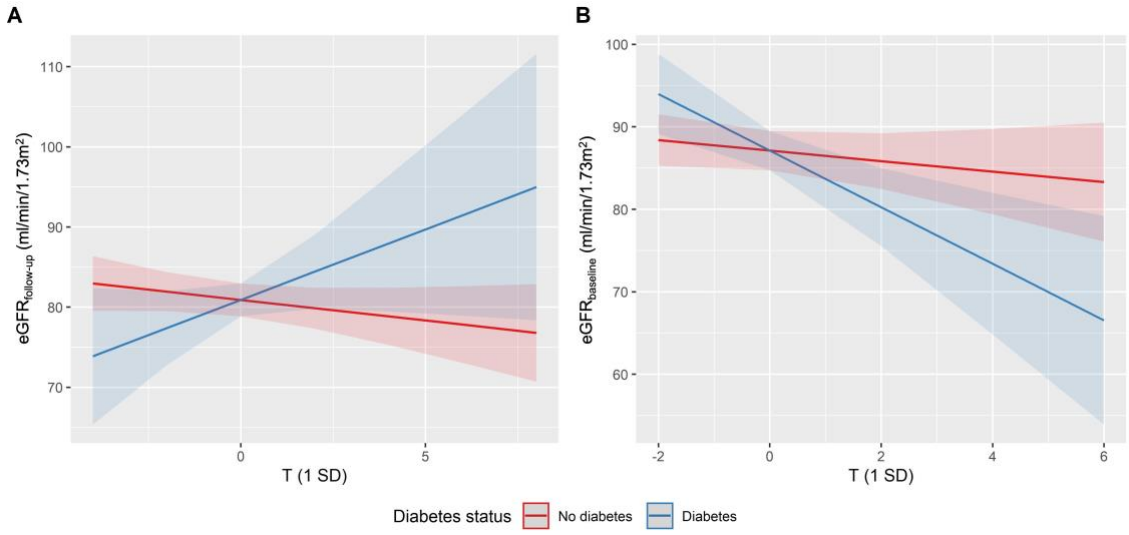

Figure 5. T levels and (A) follow-up eGFR among men and (B) baseline eGFR among women with and without T2D. (A) $P_{interaction}$ = 0.041. (B) $P_{interaction}$ = 0.014.

Abbreviations: eGFR: Creatinine-based estimated glomerular filtration rate, SD: Standard deviation, T: Testosterone.

Key findings 3 (Paper 3): In men on HD, low T levels were neither significantly associated with all-cause and CVD mortality, nor with incident CVD. However, low T levels were significantly associated with lower HRQOL. While diabetes did not modify any associations, age modified the association between low T and HUI3 scores.

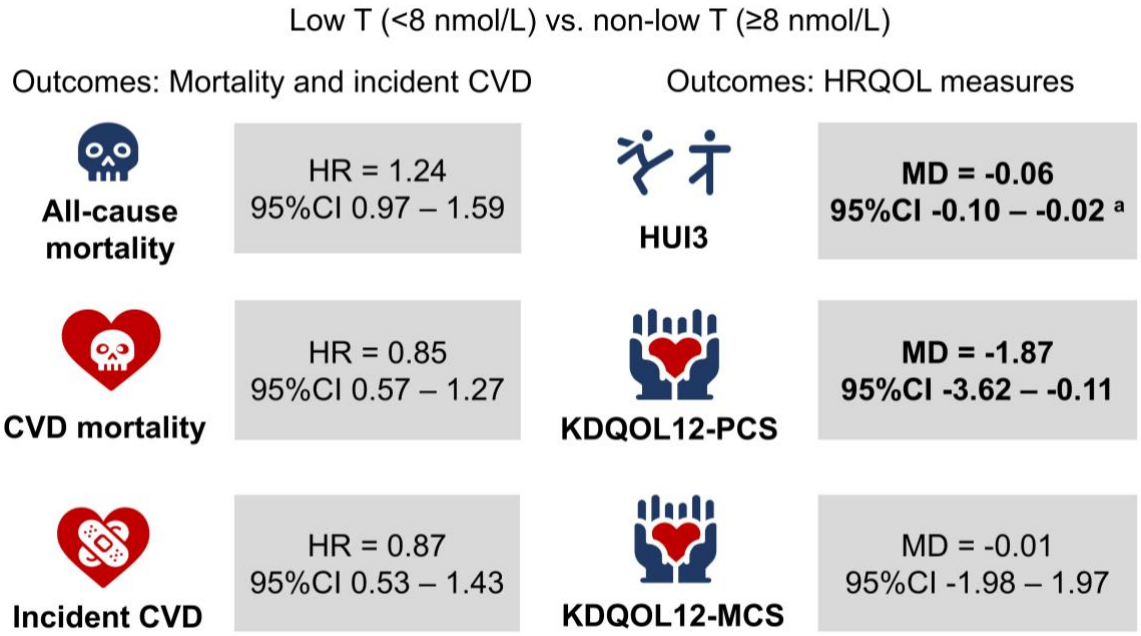

Figure 6. Result summary for Paper 3, which had a prospective study design. Bold font indicates significant results.

Abbreviations: CV: Cardiovascular, CVD: Cardiovascular disease, CI: Confidence interval, HR: Hazard ratio, HUI3: Health Utilities Index Mark 3, KDQOL12-PCS: Kidney disease quality of life physical component scores, KDQOL12-MCS: Kidney disease quality of life mental component scores, MD: Differences in means, T: Testosterone.

[a] Age was a significant effect modifier ($P_{interaction}$ = 0.04). The positive association between serum total T and HUI3 scores (MD_{HUI3}=0.04, [0.01; 0.06]). were evident only among older men (≥63 years).

Data from 587 men on HD who took part in the CKDCS were used. Over 3.7 years of median follow-up, 318 (54%) patients died. Of the patients who died, 118 deaths (37%) were CV-related. Using Cox-proportional hazard models, low T levels were not significantly associated with all-cause (HR = 1.24, [0.97; 1.59]) and CVD mortality (HR = 0.85, [0.57; 1.27]). In 289 patients without prior CV events, 82 (28%) experienced incident CVD. After accounting for death as a competing risk using Fine and Gray models, low T was not significantly associated with incident CV events (HR = 0.87, [0.53; 1.43]). Over time, patients

with low T levels had significantly lower HUI3 and KDQOL12-PCS scores (MD_{HUI3}=-0.06, [-0.10; -0.02]; $MD_{KDQOL12\text{-}PCS}$=-1.87, [-3.62; -0.11]).

Age was a significant effect modifier for the association between serum total T (continuous) and HUI3 scores: the positive association was apparent only among older men (≥63 years) (MD_{HUI3}=0.04, [0.01; 0.06]).

When serum total T was analyzed on a continuous scale, the results did not significantly change for most outcomes, except for incident CVD. Serum total T was significantly associated with an increased risk of an incident CV event (HR = 1.34, [1.07; 1.67]). All associations reported within Paper 3 were not modified by diabetes.

When we compared Bayesian information criterion (BIC) values of models that included either T and SHBG or fT, BIC values of models that included T and SHBG were persistently lower than or equal to those containing fT. Therefore, the T and SHBG model was a better fit for the CKDCS data. Consequently, fT results are not presented in this thesis but are included in Paper 3 in the appendix.

Key findings 4 (Paper 4): Among women undergoing HD, E2 levels were not linearly associated with all-cause mortality, CVD mortality, and incident CVD. However, a curvilinear association was observed only between E2 levels and all-cause mortality. Notably, compared to E2 levels in the lowest tertile, those in the highest tertile were significantly associated with higher all-cause mortality. While diabetes did not modify any associations, age modified the association between E2 levels and HUI3 scores.

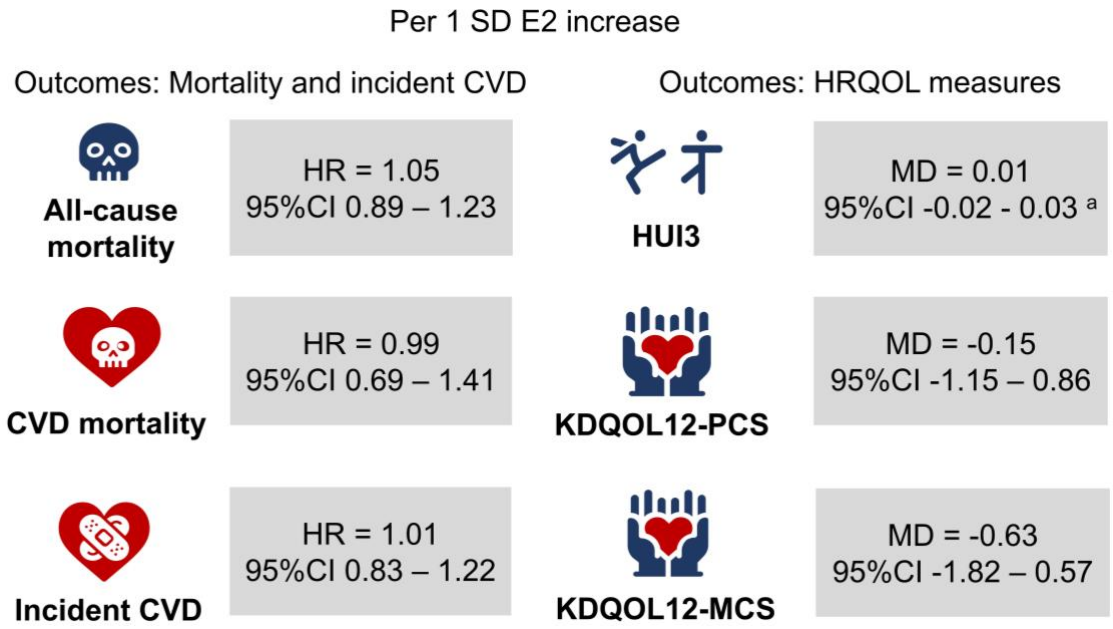

Figure 7. Result summary for Paper 4, which had a prospective design.

Abbreviations: CV: Cardiovascular, CVD: Cardiovascular disease, CI: Confidence interval, E2: Estradiol, HR: Hazard ratio, HUI3: Health Utilities Index Mark 3, KDQOL12-PCS: Kidney disease quality of life physical component scores, KDQOL12-MCS: Kidney disease quality of life mental component scores, MD: Differences in means, SD: Standard deviation.

[a] Age was a significant effect modifier ($P_{interaction}$=0.045). E2 was positively associated with HUI3 scores in younger patients (<63 years). E2 was inversely associated with HUI3 scores among older patients (≥63 years).

Data from 427 women on HD who took part in the CKDCS were used. Over a median follow-up time of 3.6 years (IQR: 1.6-7.5 years), 250 (58.6%) women died, of which 74 deaths (29.6%) were CV-related. Results from Cox proportional hazards models did not demonstrate significant associations between E2, all-cause (HR=1.05; [0.89, 1.23]) and CVD mortality (HR=0.99, [0.69, 1.41]). Out of 234 women without a prior CV event, 80 (34.2%) experienced incident CV event. We did not find significant associations between E2 and incident CVD [HR=1.01,

[0.83; 1.22]) using Fine-Gray models. Neither diabetes nor age were significant effect modifiers for the abovementioned associations.

Splines analyses suggested curvilinear associations between E2 and all-cause mortality, possibly also between E2 and CVD mortality (Section 8.4, Figure 2). Also, the Bayesian Information Criterion values of quadratic models were constantly lower than those of linear models, suggesting that a quadratic model may fit our data better. E2 was non-linearly associated with all-cause mortality ($P_{non\text{-}linear}$ = 0.022). Even though quadratic models fit our data better, we did not observe significant non-linear associations between E2, CVD mortality ($P_{non\text{-}linear}$ = 0.163), or incident CVD ($P_{non\text{-}linear}$ = 0.385). During sensitivity analysis, compared to E2 levels in the lowest tertile, E2 levels in the highest tertile were significantly associated with higher all-cause mortality (HR=1.61, 95% CI 1.16, 2.23).

Age was a significant modifier of the prospective association between E2 and HUI3 scores ($P_{interaction}$=0.045). In younger women (< 63 years), we saw a positive association between E2 levels and HUI3 scores (MD=0.032, [0.01; 0.06]). However, in older women (≥ 63 years), we observed the opposite (MD=-0.06, [-0.11; -0.01]).

We did not observe significant prospective associations between E2, KDQOL12-PCS (MD=-0.15, [-1.15; 0.86]) and KDQOL12-MCS (MD = -0.63, [-1.82; 0.57]) scores. Also, neither diabetes nor age were significant effect modifiers.

5. Discussion

The findings of this thesis suggest that men with higher E2, P4, and 17-OHP levels may have better insulin sensitivity, while women with higher E2 and 17-OHP levels may be more likely to have a worse glycemic profile. While the positive association between E2 and insulin sensitivity observed among men seems to contradict previous observations, our finding that higher fE2 (i.e. bioactive E2) was positively associated with HbA_{1c} in both men and women is in line with the current literature: high E2 could have detrimental effects on glucose metabolism. In the context of kidney function and CKD, SHBG (but not androgens) may be relevant among men, whereas among women, worse kidney function seems to be accompanied by abnormally high T levels.

While we did not observe significant associations between T, E2, and mortality, T and E2 are potentially relevant in achieving better HRQOL in HD patients. Men with low T levels are more likely to have worse HRQOL over time than their counterparts with normal T levels. Among women, the association between E2 and all-cause mortality (and possibly also between E2 and CVD mortality) may be curvilinear. However, quadratic terms of E2 were significant only for all-cause mortality, possibly due to limited sample sizes for CVD mortality. Notably, E2 levels in the highest tertile were significantly associated with higher all-cause mortality compared to E2 levels in the lowest tertile. Further, the relationship between endogenous E2 levels and HRQOL in women may depend on when HD is initiated. As E2 levels increase, younger women had better HRQOL over time, whereas older women had worse HRQOL.

Specific results have been discussed in the corresponding papers. This section highlights methodological considerations, discusses why clinical implications are still limited, and suggests possible avenues for future work.

5.1 Methodological considerations

5.1.1 Exposure, outcomes, covariables and exclusions

Sex hormone measurements were available only at baseline. Thus, sex hormone variations could not be monitored over time. Furthermore, we could not identify women with PCOS, where hyperandrogenism is a common characteristic. Thus,

we could not explicitly exclude these women. Participants taking exogenous sex hormones were excluded from the present analyses. As information regarding menstrual status was available in KORA, we were able to discern between women of different menopausal status in Papers 1 and 2.

As data linkage was possible in the CKDCS study, it allowed us to identify cases for the assessed outcomes.

However, the amount of information on covariates are finite and it was not possible to account for all possible covariates in models (due to sample size limitations). Furthermore, some participants were lost to follow-up. As such, biases can happen from unmeasured confounding, reverse causation, and/or selection bias. Furthermore, some study samples were relatively small and thus especially subgroup analyses lacked statistical power.

5.1.2 Generalizability

The majority of KORA F4, FF4, and CKDCS participants were of European descent, precluding the generalizability of our observations. As CKD progression differs by ethnic groups [102], further work is needed to ascertain the impact of ethnicity on CKD progression and associated outcomes.

5.1.3 Causal inference

The KORA F4 participants as well as the CKDCS patients were followed-up over several years which enabled us to assess the temporal sequence between exposure and outcome in the prospective analyses. However, the observational nature of the KORA and CKDCS studies precludes confirmation of causality. Mendelian Randomization (MR), a method that uses genetic variation to investigate the causal associations between exposure and outcomes in observational data [103], could be an attractive avenue to ascertain causality.

5.2 Potential implications for clinical practice

A meta-analysis of thirteen observational studies suggested that higher T levels significantly reduced T2D risk among men [104]. Further, a meta-analysis of five randomized controlled trials (mean follow-up time = 6.5 months) saw reduced fasting glucose, insulin, and triglyceride levels among hypogonadal men with T2D

treated with T [105]. A recent intervention trial showed that a two-year T treatment among 1007 men with T levels <14.0 nmol/L but without pathological hypogonadism and T2D reduced the number of participants who developed T2D was reduced in those with T intervention compared to those with lifestyle intervention [106]. Our results regarding positive associations between fE2, fasting glucose, and HbA_{1c} among women in Paper 1 agree with reports from observational studies reporting cross-sectional associations between hormonal replacement therapy (HRT) usage and slight elevations in postprandial glucose in postmenopausal women [107, 108]. However, results from other intervention studies are mixed: HRT was beneficial among postmenopausal women with T2D [109], while others reported either adverse effects [107, 108], or no effect in healthy postmenopausal women [110]. To our knowledge, the potential causal link between female sex hormones and T2D has not been studied in MR studies.

Research regarding the potential role of androgens in kidney function is still in its infancy. A handful of observational studies noted benefits on kidney function with T treatment in men [111] and male rats [112, 113]. On the other hand, experimental studies reported detrimental effects on the kidney with T treatment [31, 39, 40]. Among women, our study was the only observational study to report significant associations between androgens, kidney function, and CKD [89].

T treatment has been well-studied in the general population, but data for patients with CKD or kidney failure is scant. Although the direction of effect from Paper 3 agrees (despite the statistical insignificance) with currently available evidence that lower T levels are associated with higher mortality in men [114], and that higher T levels may be beneficial for HRQOL [115, 116], there is insufficient evidence to ascertain whether T treatment would confer beneficial effects on the kidney. A recent study reported that T treatment increased total T and fT levels up to three months and is generally well-tolerated, but no significant changes were seen beyond six and twelve months in men with CKD [117]. Not many women on HD are willing to undergo HRT [118, 119] despite positive associations between HRT usage, better HRQOL, and improved CV risk markers in women on HD with low E2 levels [120]. Also, results from observational studies (including ours) regarding associations between E2, mortality, and health-related outcomes are still inconclusive.

Collectively, the usage of sex hormone replacement to alleviate T2D and CKD symptoms remains questionable. Results from this thesis, along with the current lack of robust safety and efficacy data from interventional studies with longer follow-up times, as well as increased risk of adverse events from T replacement [121] and complexities surrounding HRT [122] precludes the recommendation for interventional studies among the general population and HD patients. Potential avenues for future research are discussed in section 6.4 below.

5.3 The emerging role of SHBG in T2D and CKD

Circulating sex hormone levels depend on SHBG levels: higher SHBG levels correspond to lower free sex hormone availability to agonize receptors, and vice versa. There is mounting suggestive evidence that higher SHBG levels, rather than sex hormones, could mitigate T2D and CKD risks.

Meta-analyses of observational studies suggest associations between low SHBG levels, higher insulin resistance [81], and increased T2D risks in men [71, 123] and women [71, 123, 124]. Similarly, results from MR analyses suggest a causal protective role of higher SHBG levels on T2D among men and women [80, 123]. Our findings in Paper 1 [88] regarding the positive association of fE2 with HbA_{1c} (among men and women) and fasting glucose (among women) are not unexpected, as lower SHBG levels can lead to higher fE2 levels.

Further MR analyses suggest that low SHBG could promote CVD among men and women [125], and higher SHBG could protect against CKD specifically among men [86]. The association between higher SHBG values (right side of the U-shaped association) and higher follow-up eGFR among men in Paper 2 [89] are partially concordant to these observations. In an apparent contrast to the aforementioned findings, a recent investigation among men on HD found that higher SHBG levels were significantly associated with higher all-cause mortality [126]. However, since weight loss can increase SHBG production by the liver [127], this contradiction could be explained by protein-energy wasting that may modify associations between risk factors and outcomes in uremic patients [10, 128].

In healthy adults, increasing SHBG levels could represent a potential strategy to prevent or delay T2D and/or CKD onset. However, more work is needed to clarify

the effects of SHBG on T2D and CKD - particularly its inter-relationship with sex hormones including specific molecular pathways involved.

5.4 Future research

Collectively, our findings provide further insight into sex-specific associations between sex hormones, SHBG, glucose- and insulin-related traits. Further, we provide possible avenues for future studies among the general population and patients with kidney failure on HD.

5.4.1 Future research in the general population

More studies with larger sample sizes are needed to corroborate our findings regarding the link between female sex hormones, glucose metabolism, and kidney function among men.

Further, androgen profiles remain unclear among women with T2D and CKD. In Paper 2, T levels were higher among women with eGFR <60 ml/min/1.73m^2 compared to those with eGFR ≥60 ml/min/1.73m^2 (0.61 vs. 0.77 nmol/L, p = 0.059). Among women with eGFR <60 ml/min/1.73m^2, those with diabetes had higher T levels than those without (0.84 vs. 0.72 nmol/L, p = 0.065). However, these differences were not statistically significant. Thus, more work is required to understand androgen profiles in women with CKD, and to further evaluate their relevance in kidney function.

5α-reductase and aromatase are enzymes involved in androgen and estrogen metabolism, respectively. 5α-reductases reduce T to DHT [129], and aromatases convert T to E2 [130]. Changes in their expression during disease states can alter sex hormone metabolism and induce physiological changes. However, changes in 5α-reductase and aromatase expression during T2D and CKD have received little attention. Compared to eugonadal men with T2D, aromatase, AR, and ER expression is lower among hypogonadal men with T2D [131]. Thus, not only is there T deficiency, mechanisms involved in sex hormone metabolism are also negatively impacted (i.e. reduced AR and ER expression) and exacerbated. Therefore, determining 5α-reductase and aromatase expression during T2D and CKD onset may provide further insight into sex hormone metabolism.

5.4.2 Future research in patients with kidney failure on HD

In men on HD, low T is common [132]. However, less is known about E2 and P4 levels in men with kidney failure. In women with kidney failure, low circulating E2 is common but T levels have been examined in only one study thus far, where Nilsson et al. reported that women with kidney failure on HD had a mean total T level of 0.9 nmol/L [126] - higher than those observed in the general population (0.58 nmol/L in Kim et al [85] and 0.62 nmol/L in Lau et al [89]). However, women included in the study of Nilsson et al [126] had moderate to severe hyperparathyroidism so its interpretation may be limited. Also, it is not known whether the elevated T levels among women in Nilsson et al [126] are of pathological significance. Due to low T and low E2 in men and women on HD, respectively, T treatment and HRT is often considered. However, in this respect, safety aspects regarding morbidity and mortality of other chronic diseases are crucial.

To date, there is no conclusive evidence that T treatment is associated with increased CV risk in hypogonadal men [133-135] and postmenopausal women [136-138]. Albeit, these findings are from underpowered trials. Further, T treatment is prescribed as a lifelong medication in men and (off-label) in women. Thus, follow-up times in available RCTs are insufficient (range: 9 months to 3 years) to determine possible long-term adverse CV effects, if any. The Testosterone Replacement therapy for Assessment of long-term Vascular Events and efficacy ResponSE in hypogonadal men (TRAVERSE) study is an ongoing study to determine CV and long-term efficacy of T supplementation in men with hypogonadism over 5 years [139]. Regardless of study outcomes, interventional studies that administer patients with exogenous sex hormones should assess kidney safety.

No studies to date assessed effects of T treatment (in men) and HRT (in women) on CV morbidity and mortality in patients with reduced kidney function. The lack of robust data causes uncertainty in the risk-benefit ratio of hormonal therapy in this high-risk population. Thus, larger studies with sufficient follow-up times and clinical outcomes are needed to better understand its safety and efficacy.

6. Conclusion

While CKD is common among patients with T2D, the prevalence, progression, and incidence of these diseases is sex-specific. Sex hormones have been postulated to mediate these sex differences. However, the etiology of T2D and CKD is multifactorial, and the relationship between sex hormones and these diseases (i.e. T2D or CKD) are bidirectional. Aberrant sex hormone levels can have adverse metabolic effects and impair kidney function. On the other hand, T2D and CKD can cause endocrinal disturbances resulting in abnormal sex hormone levels.

Although the findings supplied in this thesis are observational and some mechanisms still need to be elucidated in greater detail, we provide suggestive evidence that in the general population (1) female sex hormones are metabolically relevant in men, (2) SHBG and DHT are related with kidney function in men and women, respectively. In patients with kidney failure, the results of the current thesis suggest some associations between E2 levels and mortality among women on HD, and that low T and E2 levels could be modifiable risk factors for better HRQOL.

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Cross-sectional and prospective relationships of endogenous progestogens and estrogens with glucose metabolism in men and women: a KORA F4/FF4 Study

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ABSTRACT

Introduction Relationships between endogenous female sex hormones and glycemic traits remain understudied, especially in men. We examined whether endogenous 17α-hydroxyprogesterone (17-OHP), progesterone, estradiol (E2), and free estradiol (fE2) were associated with glycemic traits and glycemic deterioration.

Research design and methods 921 mainly middle-aged and elderly men and 390 perimenopausal/postmenopausal women from the German population-based Cooperative Health Research in the Region of Augsburg (KORA) F4/FF4 cohort study were followed up for a median of 6.4 years. Sex hormones were measured at baseline using mass spectrometry. We calculated regression coefficients (β) and ORs with 95% CIs using multivariable-adjusted linear and logistic regression models for Z-standardized hormones and glycemic traits or glycemic deterioration (ie, worsening of categorized glucose tolerance status), respectively.

Results In the cross-sectional analysis (n=1222 men and n=594 women), in men, 17-OHP was inversely associated with 2h-glucose (2hG) (β=−0.067, 95% CI −0.120 to −0.013) and fasting insulin (β=−0.074, 95% CI −0.118 to −0.030), and positively associated with Quantitative Insulin Sensitivity Check Index (QUICKI) (β=0.061, 95% CI 0.018 to 0.105). Progesterone was inversely associated with fasting insulin (β=−0.047, 95% CI −0.088 to −0.006) and positively associated with QUICKI (β=0.041, 95% CI 0.001 to 0.082). E2 was inversely associated with fasting insulin (β=−0.068, 95% CI −0.116 to −0.020) and positively associated with QUICKI (β=0.059, 95% CI 0.012 to 0.107). fE2 was positively associated with glycated hemoglobin (HbA_{1c}) (β=0.079, 95% CI 0.027 to 0.132). In women, 17-OHP was positively associated with fasting glucose (FG) (β=0.068, 95% CI 0.014 to 0.123). fE2 was positively associated with FG (β=0.080, 95% CI 0.020 to 0.141) and HbA_{1c} (β=0.121, 95% CI 0.062 to 0.180). In the sensitivity analyses restricted to postmenopausal women, we observed a positive association between 17-OHP and glycemic deterioration (OR=1.518, 95% CI 1.033 to 2.264).

Conclusions Inter-relations exist between female sex hormones and glucose-related traits among perimenopausal/postmenopausal women and insulin-related traits among men. Endogenous progestogens and estrogens appear to be involved in glucose homeostasis not only in women but in men as well. Further well-powered studies assessing causal associations between endogenous female sex hormones and glycemic traits are warranted.

Significance of this study

What is already known about this subject?

- Endogenous progesterone and estradiol (E2) were associated with type 2 diabetes (T2D) and related glycemic traits in previous cross-sectional studies in postmenopausal women.

What are the new findings?

- We demonstrated that endogenous progesterone, 17α-hydroxyprogesterone (17-OHP), the product of progesterone hydrolysis, and E2 are independently associated with glycemic traits in men as well.
- Among postmenopausal women only, we demonstrated a positive association of endogenous 17-OHP with fasting glucose and glycemic deterioration.

How might these results change the focus of research or clinical practice?

- Although regarded as female sex hormones, endogenous progestogens and estrogens appear to be involved in glucose homeostasis not only in women but in men as well.

INTRODUCTION

Extensive evidence from human and animal studies suggests that sex hormones are involved in modifying cardiometabolic risk, in particular diabetes development.[1] These differences in risk may be explained by changes in body composition, alterations in glucose metabolism, and insulin sensitivity

due to declining sex hormone concentrations associated with aging and menopause.[1] However, whether glycemic traits specifically mediate the relationship between female sex hormones and glycemic deterioration remains controversial.[2 3]

Estrogens and progestogens comprise female sex hormones. Estradiol (E2) is the most potent and abundant endogenous estrogen. Higher levels of endogenous E2 have been associated with increased type 2 diabetes (T2D) risks in several population-based settings.[4 5] Conversely, when used in hormone replacement therapy (HRT) E2 confers beneficial effects on glycemic control by reducing glycated hemoglobin (HbA_{1c}) levels,[6] fasting glucose (FG), and fasting insulin.[7] Another endogenous hormone - progesterone, important especially during pregnancy, has been found to have positive associations with FG and HbA_{1c}, and inverse associations with HOMA-β in both men and women.[8] The product of progesterone hydrolysis, 17α-hydroxyprogesterone (17-OHP), has been observed to be elevated in patients with T2D.[9] A study conducted in pregnant women showed that administration of 17-OHP caproate, a progestin-only contraceptive used to prevent preterm delivery, was associated with increased postchallenge glucose levels and increased risk of gestational diabetes (GD).[10] Notably, women who develop GD are at higher risk of developing T2D later in life.[11]

Both estrogens and progestogens exist endogenously in men as well, but they are not considered as clinically relevant as they are in women[12]—leading to the lack of studies regarding these sex hormones in men.[5] There is evidence concerning detrimental effects of estrogen deficiency in men.[13 14] However, evidence for progestogens is limited.[8 15] Available studies involving endogenous estrogen are mainly cross-sectional, have limited sample sizes, and lack comprehensive glycemic outcomes. Additionally, we are not aware of any epidemiological study to date investigating endogenous 17-OHP as an exposure.

Therefore, this study was conducted to explore the associations of endogenous 17-OHP, progesterone, E2, and free estradiol (fE2) with FG, 2h-glucose (2hG), HbA_{1c}, fasting insulin, and Quantitative Insulin Sensitivity Check Index (QUICKI), separately in men and in perimenopausal/postmenopausal women. Furthermore, we examined prospective associations of these female sex hormones with glycemic deterioration defined as aberrant progressions from NGT or pre-diabetes to either pre-diabetes or diabetes during 6.4 years of follow-up.

METHODS

Study population and selection criterions

The data for the study were obtained from the Cooperative Health Research in the Region of Augsburg (KORA) baseline (F4) (2006–2008) and follow-up (FF4) studies (2013–2014). Both studies are follow-up examinations of the KORA S4 study (1999–2001) conducted in Augsburg, Southern Germany, and two surrounding counties. The

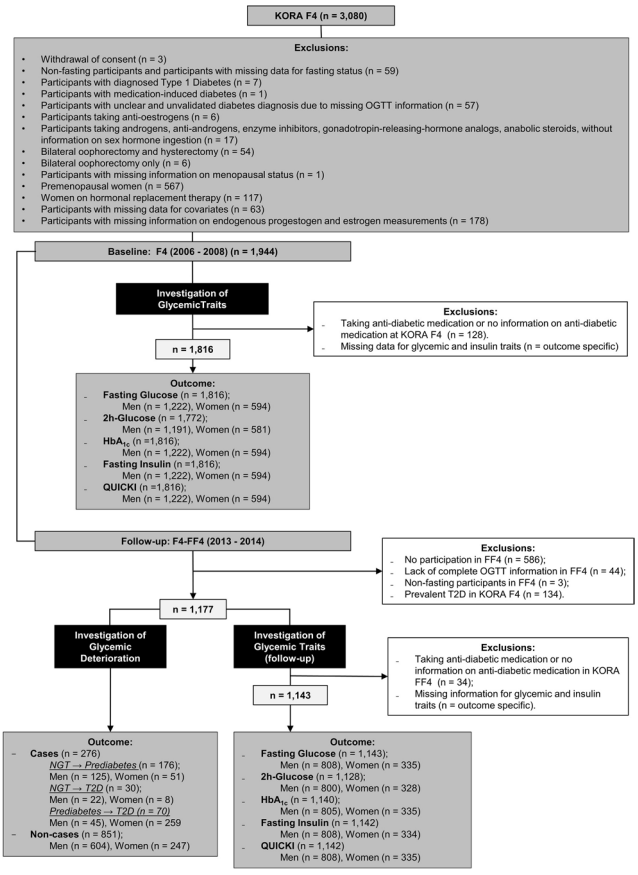

Figure 1 Flowchart showing sample sizes and exclusions. HbA_{1c}, glycated hemoglobin; KORA, Cooperative Health Research in the Region of Augsburg; NGT, normoglycemia; OGTT, oral glucose tolerance test; QUICKI, Quantitative Insulin Sensitivity Check Index; T2D, type 2 diabetes.

study design has been described previously in detail.[16] The KORA F4 study included 3080 participants aged between 32 and 81 years, of whom 2161 also participated in KORA FF4. Three participants who withdrew consent were removed from the analyses. After further exclusions as described in figure 1, the final sample for the cross-sectional analysis comprised 1816 participants (1222 men and 594 women), while the prospective analysis sample comprised 1311 participants (921 men and 390 women). Participants taking antidiabetic medications were excluded from both cross-sectional and prospective analyses examining continuous glycemic traits as outcomes.

Assessment of the outcomes

Previously known T2D was a self-report that could be validated by a physician or medical chart review, or as self-reported current use of glucose-lowering medication. Participants without known T2D were given a standard 75 g, oral glucose tolerance test (OGTT). Blood samples were taken without stasis after an overnight fast of ≥8 hours and 2 hours after glucose solution ingestion. Serum glucose was measured using hexokinase-G6PD (GLUFlex; Dade Behring, USA). In KORA FF4, glucose levels were quantified in serum either by using the glucose colorimetric assay (Dimension Vista 1500

System; Siemens Healthcare Diagnostics, USA) or the GLUC3 assay (Cobas c702; Roche Diagnostics GmbH, Germany). No calibration was needed for glucose as the double measurements were very similar. Normoglycemia (NGT) (ie, FG <6.1 mmol/L and 2hG <7.8 mmol/L), pre-diabetes (FG ≥6.1 mmol/L but <7.0 mmol/L, and 2hG <7.8 mmol/L (isolated impaired fasting glucose (IFG)) or FG of <6.1 mmol/L and 2hG ≥7.8 mmol/L but <11.1 mmol/L (isolated impaired glucose tolerance (IGT)), or both (IFG and IGT)), and newly-diagnosed diabetes (FG ≥7.0 mmol/L or 2hG ≥11.1 mmol/L) were defined according to the 1999/2006 WHO criteria.[17] In KORA F4, HbA_{1c} was quantified in hemolysed whole blood using cation-exchange high-performance liquid chromatography (HPLC) (Adams HA 8160 Hemoglobin Analysis System; A. Menarini Diagnostics, Italy). In KORA FF4, HbA_{1c} concentrations were determined using ion-exchange HPLC (Variant II Turbo HbA_{1c} Kit; Bio-Rad Laboratories, USA). In KORA F4, fasting insulin was measured in thawed serum by an elctrochemiluminescence immunoassay (Cobas e602 Immunoassay Analyser; Roche Diagnostics GmbH, Germany). In KORA FF4, fasting insulin was quantified using either solid phase enzyme-labeled chemiluminescent immunometric assay (Immulite 2000 Systems Analyser, Siemens) or electrochemiluminescence immunoassay (Cobas e602 Immunoassay Analyser; Roche Diagnostics GmbH, Germany). Due to the change in measurement instruments and assays in KORA FF4, calibration was required for insulin measurements. This has been described previously in detail.[18] QUICKI was used as a measure of insulin sensitivity and was calculated using the following formula: $QUICKI=1/(\log_{10}(FG)+\log_{10}(\text{fasting insulin}))$, with FG in milligram per decilitre and fasting insulin in microunit per millilitre. Glycemic deterioration was defined as the transition from NGT to pre-diabetes, NGT to T2D, and pre-diabetes to T2D from F4 to FF4. For this investigation, 135 participants with prevalent T2D at F4 were excluded, leading to a final sample for this analysis of 851 non-cases and 278 cases (online supplemental figure 1).

Assessment of the exposures: sex hormone measurements

Progesterone, 17-OHP, and E2 were quantified in serum using liquid chromatography–electrospray ionization–tandem mass spectrometry and the Absolute*IDQ* Stero17 Kit (BIOCRATES Life Sciences, Austria) (online supplemental material 1).[19] The calibration, imputation, and normalization of sex hormone measurements are described in detail in online supplemental material 2. fE2 concentrations were estimated based on measured sex hormone-binding globulin (SHBG), E2, and albumin using the formula derived by Rinaldi *et al*[20] (online supplemental material 3). SHBG in serum was quantified using the ARCHITECT SHBG assay, a chemiluminescent microparticle immunoassay (Abbott Laboratories, USA). Albumin in serum was quantified using immunonephelometry (ALB Flex; Dade Behring, Germany).

Assessment of covariates

In KORA F4, total cholesterol and high-density lipoprotein (HDL) cholesterol were measured in fresh serum by enzymatic methods (CHOL Flex and AHDL Flex, Dade Behring). Triglycerides were measured in fresh serum enzymatically (glycerine phosphate oxidase peroxidase method) (TGL Flex, Dade Behring). C reactive protein (CRP) was quantified from frozen plasma using a high-sensitivity latex-enhanced nephelometric assay (BN II Analyzer, Dade Behring). Thyroid-stimulating hormone (TSH) was measured using electrochemiluminescent methods (Dimension Vista Systems; Siemens, Germany). Serum creatinine was measured in fresh serum with a modified Jaffe test (KREA Flex, Dade Behring) according to IDMS standards. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[21] Information on age, sex, statin medication, hypertension, smoking status, alcohol consumption, physical activity, and history of parental diabetes was assessed using a standardized interview, performed by trained medical staff. Hypertension was defined as having a blood pressure of >140/90 mm Hg or taking antihypertensive medication, given that the participants were aware of having hypertension. Information on medication use within 7 days before examination was obtained from a database.[22] Smoking status was categorized as never smoked, former smokers, and current smokers (smoking≥1 cigarette a day). Alcohol consumption was categorized into three groups: no consumption (0 g/day), moderate consumption (men 0.1–29.9 g/day and women 0.1–19.9 g/day), and high consumption (men≥40 g/day and women≥20 g/day). Physical activity was estimated through two separate four-category interview questions regarding the time spent per week on sports activities in summer and winter. Possible answers were (1)>2 hours, (2) 1–2 hours, (3)<1 hour, and (4) none. Participants who had a total score of <5, obtained by summing the numbers (1)–(4) relating to winter and summer, were classified to be 'physically active'.[23] Parental diabetes was categorized as no parental diabetes history, unknown parental diabetes history, or ≥1 parent with diabetes history.

Statistical analyses

Baseline characteristics of normally distributed continuous covariates are expressed as means with corresponding SD. Non-normally distributed continuous covariates were expressed as medians with the corresponding 25th and 75th percentiles. Proportions are expressed as percentages. Differences between participants with and without glycemic deterioration were calculated using Mann-Whitney U tests, while differences in categorical variables were compared using Kruskal-Wallis tests. Skewed variables were natural log (ln)-transformed to improve normalization. Z-standardization was performed sex-specifically for exposures, respectively, to achieve comparability despite their different scales. Due to significant

interactions between sex and some hormones regarding glycemic traits (online supplemental table 1), sex-stratified analyses were employed throughout this study.

Linear regression was performed to explore the cross-sectional and prospective relationships between progestogens and estrogens with glycemic traits, such as FG, 2hG, HbA_{1c}, fasting insulin, and QUICKI. β-estimates with 95% CIs for Z-scores of sex hormones are given as per one sex-specific SD increase in ln-transformed progestogens and estrogens, respectively. Association analyses focusing on pathophysiological mechanisms were adjusted for F4 T2D risk factors such as age, waist circumference, height, ln(triglycerides), total cholesterol:HDL cholesterol ratio, actual hypertension (yes/no), and use of statins (yes/no) (model 1). Additional adjustments included lifestyle risk factors such as smoking status (never/former/current), alcohol consumption (no/low/high), and physical activity (active/inactive), and additionally, ln(CRP) (continuous), ln(TSH) (continuous), eGFR (continuous), and history of parental diabetes (no history/unknown history/≥1 parent with diabetes) (model 2). In the prospective analyses, there were further adjustments for F4 values of respective glycemic traits.

We calculated ORs with 95% CIs using logistic regression to investigate associations between female sex hormones and glycemic deterioration. These associations were additionally investigated for non-linearity by testing whether the introduction of a restricted cubic spline, with three knots placed at the 30th, 60th, and 90th percentiles, would improve the model fit where medians were set as the reference values for each exposure.

The confounders that constitute our models are common T2D risk factors, along with variables that affect T2D pathophysiology and circulating sex hormone levels. We adjusted for statin usage as they can increase T2D risks.[24] TSH was adjusted due to its impact on sex hormone metabolism.[25] We performed several sensitivity analyses: (1) further adjusting models containing E2 as the exposure for SHBG as SHBG determines circulating fE2 levels,[26] (2) further adjusting models with progesterone as the exposure for albumin as it binds extensively to albumin,[27] (3) excluding perimenopausal women (n=66) as sex hormone fluctuates during perimenopause. Given the homogeneity of progestogens, interaction analyses between 17-OHP and progesterone were performed where significant associations were present to determine whether combinations of different progestogen concentrations would influence the outcomes. The interaction effects are presented using contour plots. Significance levels were based on two-sided tests, where p values of ≤0.05 were considered statistically significant. Statistical analyses were performed using R V.3.6.1.

RESULTS

Baseline characteristics

Men and women with glycemic deterioration (ie, cases) were older; had larger waist circumference and higher triglyceride levels and total cholesterol:HDL cholesterol ratio; were more likely to be hypertensive; had elevated CRP; and were more likely to have ≥1 parent with diabetes compared with those without glycemic deterioration (ie, non-cases). Among women, cases had lower TSH levels. In men, cases had higher 17-OHP, E2, and fE2 levels compared with non-cases. In women, sex hormone levels were not significantly different between cases and non-cases. At F4 and FF4, cases had higher FG, 2hG, HbA_{1c}, fasting insulin, and lower QUICKI values compared with non-cases in men and women (table 1).

Cross-sectional associations of endogenous progestogens and estrogens with glycemic traits

Cross-sectional associations are summarized in figure 2. In men, 17-OHP was inversely associated with 2hG (β=−0.074, 95% CI −0.130 to −0.019), fasting insulin (β=−0.093, 95% CI −0.140 to −0.046), and positively associated with QUICKI (β=0.079, 95% CI 0.032 to 0.126) after adjustment using model 1. On further adjustment (model 2), the significance persisted for all three outcomes: 2hG (β=−0.067, 95% CI −0.120 to −0.013), fasting insulin (β=−0.074, 95% CI −0.118 to −0.030), and QUICKI (β=0.061, 95% CI 0.018 to 0.105). Inverse associations were detected between progesterone and fasting insulin in model 1 (β=−0.052, 95% CI −0.096 to −0.008). The association remained significant after further adjustment for T2D risk factors (model 2: β=−0.045, 95% CI −0.086 to −0.004) and additional adjustment for albumin (β=−0.047, 95% CI −0.088 to −0.006). Also, progesterone was initially associated with QUICKI in model 1 (β=0.045, 95% CI 0.001 to 0.088), but the association became non-significant after further adjustment (model 2, β=0.040, 95% CI −0.001 to 0.080) (online supplemental table 2). In women, 17-OHP was positively associated with fasting glucose (β=0.071, 95% CI 0.015 to 0.127) in model 1. The significance persisted after further adjustment in model 2 (β=0.068, 95% CI 0.014 to 0.123). No further associations were found between 17-OHP and progesterone and glycemic traits in women (online supplemental table 3).

In men, after adjustment using model 1, E2 was inversely associated with 2hG (β=−0.059, 95% CI −0.118 to −0.001), fasting insulin (β=−0.113, 95% CI −0.163 to −0.062), and positively associated with QUICKI (β=0.105, 95% CI 0.054 to 0.155). After further adjustment in model 2, significant associations ceased for 2hG (β=−0.024, 95% CI −0.081 to 0.033), while it persisted for fasting insulin (β=−0.068, 95% CI −0.116 to −0.020) and QUICKI (β=0.059, 95% CI 0.012 to 0.107). On further adjustment with SHBG, the associations of E2 with 2hG (β=−0.013, 95% CI −0.073 to 0.046) and fasting insulin (β=−0.055, 95% CI −0.105 to −0.005) did not change significantly. However, the association between E2 and QUICKI ceased (β=0.044, 95% CI −0.005 to 0.093). fE2 was found to be positively associated with HbA_{1c} after adjustment in models 1 (β=0.012, 95% CI 0.004 to 0.021) and 2 (β=0.079, 95% CI 0.027 to 0.132). No further associations were found between fE2 and glycemic traits in men. In women, no

Table 1 Descriptive characteristics of men and perimenopausal/postmenopausal women in KORA F4/FF4*

	Men (n=796)			Perimenopausal/postmenopausal women† (n=331)		
	Non-cases‡ (n=604)	Cases‡ (n=192)	P value	Non-cases‡ (n=247)	Cases‡ (n=84)	P value
Age (years)	51.6 (12.2)	58.5 (10.9)	<0.001	59.7 (8.5)	62.4 (8.5)	0.005
Height (cm)	177 (6.9)	175 (6.9)	<0.001	161 (6.1)	160 (6.4)	0.068
Waist circumference (cm)	95 (89,103)	101 (95,109)	<0.001	85 (78,93)	93 (88,102)	<0.001
Triglycerides (mmol/L)	1.26 (0.87,1.74)	1.56 (1.07, 2.4)	<0.001	1.03 (0.75,1.39)	1.24 (0.99,1.78)	<0.001
Total cholesterol/HDL cholesterol	4.17 (3.51,5.00)	4.54 (3.85,5.47)	<0.001	3.60 (3.02,4.19)	4.10 (3.47,4.78)	<0.001
Hypertension (%)	28.6	50.0	<0.001	28.3	52.3	<0.001
Statin use (%)	6.9	17.7	<0.001	10.5	9.5	0.957
Smoking status						
Never (%)	32.9	37.5	0.081	54.3	63.1	0.306
Former (%)	46.4	49.0		31.2	27.4	
Current (%)	20.7	13.5		14.6	9.5	
Alcohol consumption						
None (%)	16.4	20.3	0.435	35.2	45.2	0.258
Moderate (%)	66.2	64.1		49.8	42.9	
High (%)	17.4	15.6		14.9	11.9	
Physically active (%)	59.6	57.3	0.629	63.9	51.2	0.052
CRP (mg/L)	0.85 (0.44,1.78)	1.28 (0.67, 2.28)	<0.001	1.05 (0.53,2.05)	1.96 (1.02,4.06)	<0.001
eGFR (mL/min/1.73 m^2)	92.1 (14.9)	86.3 (13.3)	<0.001	86.2 (14.5)	84.5 (14.7)	0.354
TSH (mIU/L)	1.25 (0.87,1.85)	1.36 (0.91, 1.99)	0.085	1.32 (0.87,1.88)	1.18 (0.75,1.65)	0.038
Parental history of diabetes (%)						
Both parents without diabetes	65.9	48.9	<0.001	60.3	47.6	0.091
Unknown parental history	14.1	26.6		16.2	17.8	
≥1 parent with diabetes	20.0	24.5		23.5	34.5	
17-OHP (nmol/L)	2.88 (2.12,3.94)	2.51 (1.93,3.52)	0.002	0.77 (0.52,1.21)	0.87 (0.52,1.34)	0.267
Progesterone (nmol/L)	0.20 (0.12,0.32)	0.17 (0.10,0.31)	0.063	0.12 (0.04,0.23)	0.12 (0.06,0.19)	0.762
E2 (nmol/L)	0.49 (0.36,0.68)	0.42 (0.29,0.54)	<0.001	0.17 (0.09,0.28)	0.17 (0.10, 0.27)	0.970
fE2 (nmol/L)	0.010 (0.007,0.016)	0.008 (0.006, 0.013)	<0.001	0.003 (0.001,0.004)	0.003 (0.001, .005)	0.406
F4						
FG (mmol/L)	5.22 (4.94,5.50)	5.61 (5.33,5.89)	<0.001	5.00 (4.78,5.33)	5.42 (5.17,5.94)	<0.001
2hG (mmol/L)	5.50 (4.67,6.33)	6.61 (5.71,7.40)	<0.001	5.39 (4.56,6.47)	6.72 (5.77,7.40)	<0.001
HbA_{1c} (%)	5.3 (5.1,5.5)	5.5 (5.4,5.8)	<0.001	5.4 (5.3,5.6)	5.6 (5.4, 5.9)	<0.001

Continued

Table 1 Continued

	Men (n=796)			Perimenopausal/postmenopausal women† (n=331)		
	Non-cases‡ (n=604)	Cases‡ (n=192)	P value	Non-cases‡ (n=247)	Cases‡ (n=84)	P value
Fasting insulin (pmol/L)	50.0 (36.0,66.0)	66.0 (49.0,102.0)	<0.001	46.2 (35.4,66.0)	66.0 (47.3,90.0)	<0.001
QUICKI	0.35 (0.028)	0.33 (0.028)	<0.001	0.35 (0.023)	0.33 (0.026)	<0.001
FF4						
FG (mmol/L)	5.44 (5.16,5.72)	6.22 (5.77,6.55)	<0.001	5.27 (4.94,5.61)	6.05 (5.55,6.38)	<0.001
2hG (mmol/L)	5.52 (4.66, 6.50)	8.38 (7.33,9.99)	<0.001	5.66 (4.72,6.49)	8.27 (7.77,10.1)	<0.001
HbA_{1c} (%)	5.4 (5.1,5.5)	5.6 (5.4,6.0)	<0.001	5.4 (5.3,5.6)	5.7 (5.4,5.9)	<0.001
Fasting insulin (pmol/L)	49.9 (36.6,74.9)	81.0 (56.5,117.6)	<0.001	52.2 (36.5,74.9)	84.0 (58.6,105.3)	<0.001
QUICKI	0.34 (0.029)	0.32 (0.029)	<0.001	0.35 (0.029)	0.32 (0.024)	<0.001

*Men and perimenopausal/postmenopausal women not taking antidiabetic medication.
†Perimenopausal/postmenopausal women not on oral contraceptives or HRT.
‡Comparison of descriptive characteristics of the study population with (case) and without (non-case) glycemic deterioration.Glycemic deterioration (yes/no) is defined as the progression from NGT to pre-diabetes, NGT to T2D, and pre-diabetes to T2D from F4 to FF4.
CRP, C reactive protein; E2, Estradiol; eGFR, Estimated glomerular filtration rate (creatinine-based); F4, baseline; fE2, Free estradiol; FF4, follow-up; FG, Fasting glucose; HbA_{1c}, Glycated hemoglobin; HDL, High-density lipoprotein; 2hG, 2h-glucose; HRT, Hormone replacement therapy; KORA, Cooperative Health Research in the Region of Augsburg; NGT, Normoglycemia; 17-OHP, 17α-hydroxyprogesterone; QUICKI, Quantitative Insulin Sensitivity Check Index; T2D, type 2 diabetes; TSH, Thyroid-stimulating hormone.

significant associations were observed between E2 and glycemic traits after adjustment in models 1 and 2, and after further adjustment for SHBG. However, fE2 was positively associated with fasting glucose after adjusting with models 1 and 2, respectively (model 2: β=0.080, 95% CI 0.020 to 0.141) and HbA_{1c} (model 2: β=0.121, 95% CI 0.062 to 0.180) (online supplemental table 3). Substitution of waist circumference and height with body mass index did not significantly change the results (data not shown).

In the sensitivity analyses, among men, the inverse association between progesterone and fasting insulin remained significant in model 2 after additional adjustment for albumin. As for the association between progesterone and QUICKI, additional adjustment for albumin in model 2 reinstated the significance (β=0.041, 95% CI 0.001 to 0.082), which was previously made insignificant after adjustment in model 2 (β=0.040, 95% CI −0.001 to 0.082) (online supplemental table 2). The positive association between E2 and QUICKI remained significant after additional adjustment with SHBG in model 2. In women, additional adjustments with albumin and SHBG did not significantly change the results (online supplemental table 3). After perimenopausal women were excluded, associations between sex hormones and fasting glucose, as well as HbA_{1c}, generally became stronger. Specifically, progesterone (β=0.071, 95% CI 0.007 to 0.136) and E2 (β=0.076, 95% CI 0.014 to 0.137) became significantly associated with fasting glucose and progesterone with HbA_{1c} (β=0.071, 95% CI 0.008 to 0.133) (online supplemental table 3).

In men, there were interactions between 17-OHP and progesterone (online supplemental table 6). Selected results are shown in figure 3. Lower fasting insulin levels were observed when both 17-OHP and progesterone levels were at the lowest or highest (figure 3A). Higher QUICKI values were observed in men when both 17-OHP and progesterone concentrations were at the lowest or highest. Lower QUICKI values were observed in men with the highest progesterone and lowest 17-OHP levels and also with the highest 17-OHP and lowest progesterone levels (figure 3B). In women, no interactions were detected between 17-OHP and progesterone on fasting glucose (online supplemental table 6).

Glycemic deterioration

No significant associations between progestogens and estrogens with glycemic deterioration were observed in men and women (figure 4). After removal of perimenopausal women in the sensitivity analysis, 17-OHP was significantly associated with glycemic deterioration in postmenopausal women (OR=1.518, 95% CI 1.033 to 2.264)) (online supplemental table 4). We also assessed for non-linear relationships across different progestogen and estrogen concentrations (online supplemental figure 2). However, there were no indications for significant non-linear relationships (online supplemental table 5).

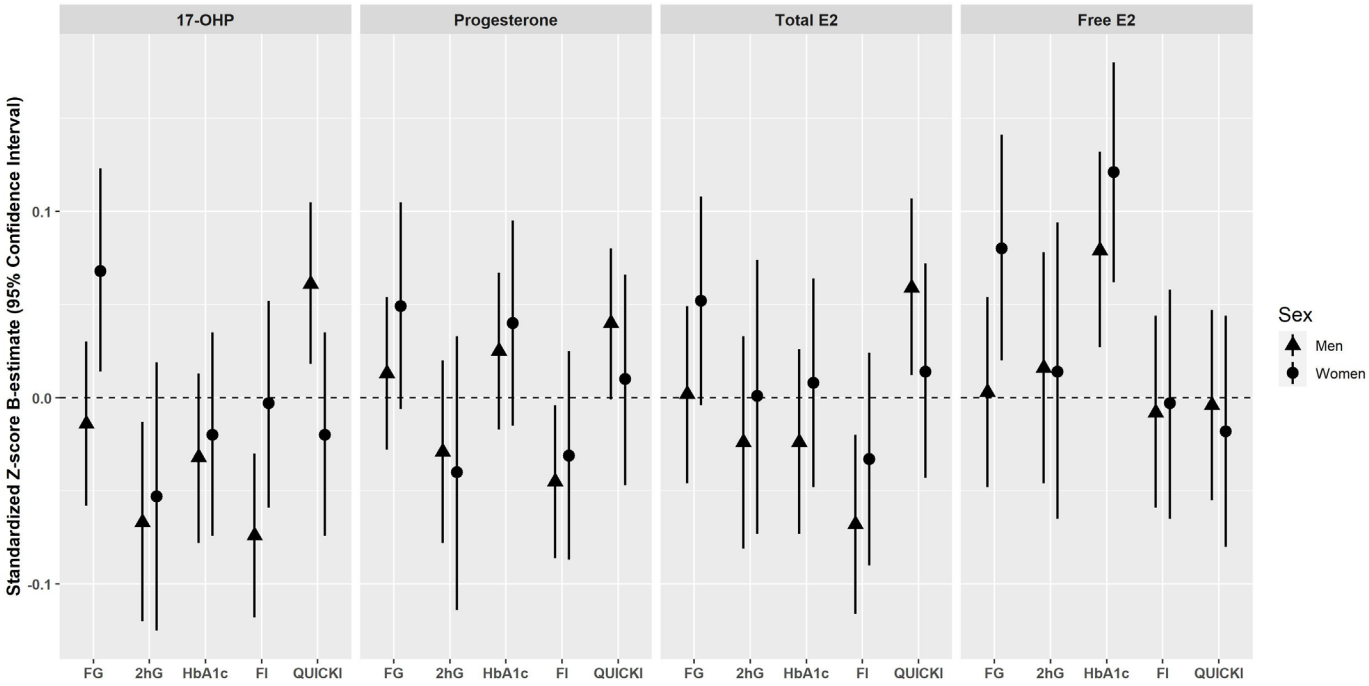

Figure 2 Cross-sectional associations of endogenous progestogens and estrogens with glycemic traits in men and women of the KORA F4 cohort*. Results are expressed as the change in 1 log unit of the continuous outcome (standardized Z-score β-estimate with 95% CI) per 1 sex-specific SD increase in the respective progestogens and estrogens adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol:high-density lipoprotein cholesterol ratio, hypertension, statin use, smoking status, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental history of diabetes (model 2). *Men and perimenopausal/postmenopausal women who did not take antidiabetic medication. CRP, C reactive protein; E2, Estradiol; eGFR, estimated glomerular filtration rate; F4, baseline; FG, fasting glucose; FI, fasting insulin; HbA_{1c}, glycated hemoglobin; 2hG, 2h-glucose; KORA, Cooperative Health Research in the Region of Augsburg; 17-OHP, 17α-hydroxyprogesterone; QUICKI, Quantitative Insulin Sensitivity Check Index; TSH, thyroid-stimulating hormone.

Prospective associations of endogenous progestogens and estrogens with glycemic traits

In men, progesterone was positively associated with fasting insulin (β=0.052, 95% CI 0.005 to 0.098) and inversely associated with QUICKI (β=−0.048, 95% CI −0.095 to

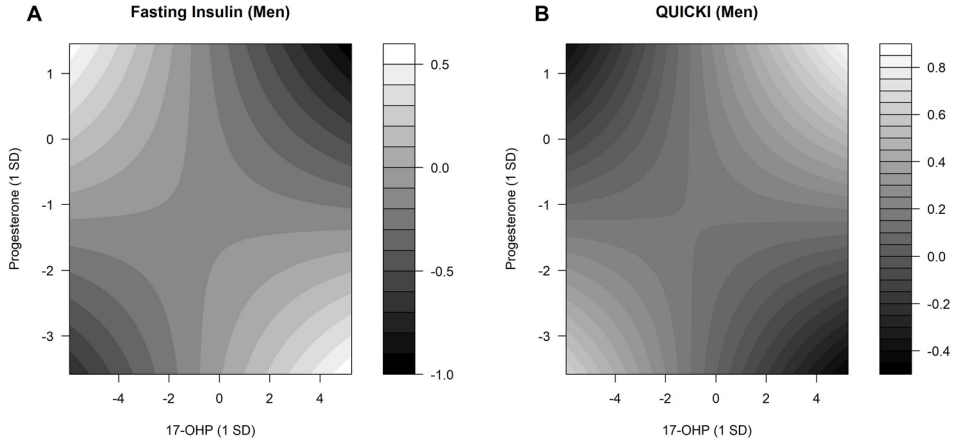

Figure 3 Interaction effects between 17-OHP and progesterone regarding fasting serum insulin and QUICKI. Contour plots estimated by linear regression models demonstrate the changes in fasting insulin and QUICKI for different concentrations of 17-OHP and progesterone. The predicted fasting serum insulin and QUICKI values were presented with gradients, ranging from black (low fasting insulin and QUICKI values) to white (high fasting insulin and QUICKI values). (A) P value for interaction=0.002. (B) P value for interaction=0.011. Linear predictions were adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol:high-density lipoprotein cholesterol ratio, hypertension, statin use, smoking status, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental diabetes history. 17-OHP, 17α-hydroxyprogesterone; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; QUICKI, Quantitative Insulin Sensitivity Check Index; TSH, thyroid-stimulating hormone.

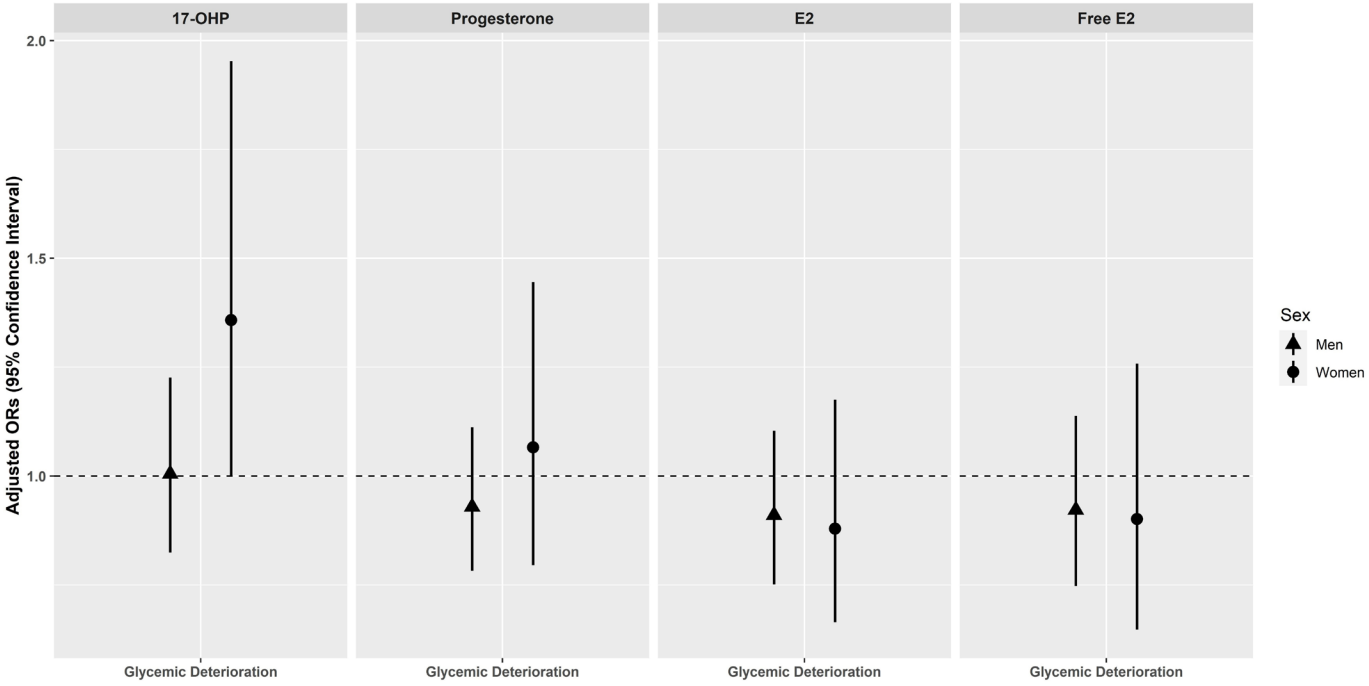

Figure 4 Association of endogenous progestogens and estrogens with glycemic deterioration in men and women of the KORA F4/FF4 cohort*. Adjusted ORs with 95% CIs for glycemic deterioration per 1 sex-specific SD increase in log-transformed progestogen and estrogen. ORs are adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol:high-density lipoprotein cholesterol ratio, hypertension, statin use, smoking status, alcohol consumption, physical activity, CRP, eGFR (creatinine-based), TSH, and parental history of diabetes (model 2). *Men and perimenopausal/postmenopausal women without prevalent type 2 diabetes at baseline. CRP, C reactive protein; E2, Estradiol; eGFR, estimated glomerular filtration rate; F4, baseline; FF4, follow-up; KORA, Cooperative Health Research in the Region of Augsburg; 17-OHP, 17α-hydroxyprogesterone; TSH, thyroid-stimulating hormone.

−0.000) after adjustment in model 1. However, associations between progesterone and fasting insulin (β=0.044, 95% CI −0.002 to 0.091) and QUICKI (β=−0.040, 95% CI −0.088 to 0.007) ceased after adjustment in model 2 (online supplemental table 7). In women, no associations were found between progestogens and estrogens and glycemic traits regardless of adjustments in models 1 and 2 and further adjustments for SHBG and albumin (online supplemental table 8).

DISCUSSION

In this population-based study of mainly middle-aged and elderly participants, we found that progestogens and estrogens were associated with glucose and insulin traits in men, whereas in women, associations were found only with glucose traits. Specifically, in the cross-sectional analyses in men, we found that higher levels of 17-OHP, progesterone, and E2 were associated with lower fasting insulin, whereas higher 17-OHP and E2 were associated with higher QUICKI values. Concerning glucose traits among men, higher 17-OHP levels were associated with lower 2hG concentrations whereas higher fE2 levels were associated with higher HbA_{1c} concentrations. Among women, positive associations were observed between 17-OHP and fasting glucose and between fE2 and fasting glucose as well as HbA_{1c}. After exclusion of perimenopausal women, we observed significant associations of progesterone, 17-OHP and E2 with fasting glucose and of progesterone with HbA_{1c}. Furthermore, we found significant interactions between 17-OHP and progesterone on fasting insulin levels and QUICKI in men. In the prospective analyses, we found no associations in both men and women after multivariable adjustment in the main analyses. However, in the sensitivity analysis, the exclusion of perimenopausal women revealed that postmenopausal women with elevated baseline 17-OHP levels had an increased risk of glycemic deterioration.

Congruent to our results, a cross-sectional study conducted in a rural Chinese population found positive associations of progesterone with fasting glucose, HbA_{1c}, and an increased risk of prevalent pre-diabetes and T2D in men and women.[8] Furthermore, in the study of Jiang *et al*[8] in men and women, progesterone was inversely associated with HOMA-2β, an index of β-cell function, but not with fasting insulin as seen among men in the present study. The slightly diverging observations could be due to differences in ethnicity, lifestyle factors, socioeconomic status, and sample size between the populations. A recent study in men and women by Lu *et al*[9] reported positive correlations between 17-OHP and fasting glucose, 2hG, and HbA_{1c}. This was consistent with our observations of a positive association between fasting glucose and 17-OHP among women. However, the study by Lu *et al*[9] performed correlation analyses without appropriate

confounder adjustments, therefore limiting its interpretability. A Swedish longitudinal study (n=240) conducted among opposite-sex twins found no association between progesterone and diabetes risk.[15] This corresponds to our null findings regarding the association of progestogens with glycemic deterioration. In the present study, the cross-sectional and prospective effect estimates of progesterone on fasting insulin and QUICKI show a change of direction in men. This could be due to the presence of (negative) confounding or random chance (given the insignificant results of model 2). However, our cross-sectional results are in line with current experimental evidence as described further.

Mechanisms by which progestogens alter glucose and insulin metabolism are nebulous, but there are some possible explanations. Elevated 17-OHP can induce hyperglycemia in female mice, and CYP17A1 is suggested to play a role in modulating this effect.[9] CYP17A1 converts progesterone to 17-OHP,[28] and Lu *et al*[9] proposed that increased 17-OHP levels due to aberrant expression of CYP17A1 in obese mice increase blood glucose via the glucocorticoid (GC) receptor. GCs can confer hyperglycemia and gluconeogenesis[29] and could explain the positive association between 17-OHP and fasting glucose in women. However, in men, we saw that 17-OHP levels were negatively associated with 2hG levels. Among men, higher 17-OHP levels could improve insulin sensitivity, thus lowering glucose levels. Specific variants in genes coding for CYP17A1 were suggestive of T2D susceptibility. Wang *et al*[30] showed that polymorphism rs12413409, corresponding to CYP17A1 under-expression, was associated with increased fasting glucose only in men. Hence, the role of the polymorphism in glucose metabolism specific to men could explain our observations. We also observed interactions between 17-OHP and progesterone on fasting insulin in men. Imbalanced progestogen concentrations can cause aberrant GC receptor signaling due to competitive binding[31] and may thereby contribute to suboptimal insulin levels. Consequently, perturbations in glucose homeostasis may arise. Until now, 17-OHP and diabetes risk have been implicated only in pregnant women.[10] However, we showed that increased endogenous 17-OHP could also impact glucose homeostasis later in life among postmenopausal women. Fluctuating sex hormones during the cycle in perimenopausal women[32] could have confounded our results when perimenopausal and postmenopausal women were analyzed together.

In men, E2 was negatively associated with fasting insulin levels and positively with insulin sensitivity in our study. Our observations are consistent with a study by Yan *et al*,[33] where they found that treatment with E2 improves insulin sensitivity in hepatocytes. A Mendelian randomization study by Wang *et al*[34] found a causative protective role of SHBG against T2D. However, weaker causal estimates of the causative protective role of SHBG compared with those observed from meta-analyses of prospective studies suggest that the observed protective role of SHBG could be confounded, as opposed to direct SHBG action. This is consistent with our results as we saw that the positive associations between E2 and insulin sensitivity were independent of SHBG and typical T2D risk factors. Our results showed persistent positive associations between fE2 and HbA_{1c} in both men and women. fE2 is the portion of E2 that is not bound to SHBG and is free to activate estrogen receptors (ERs). Under normal circumstances, E2 suppresses hepatic gluconeogenesis, potentially mediated through the activation of ERα-phosphoinositide 3-kinase-Akt-Foxo1 signaling.[33] Due to the age-related E2 decline in both men and postmenopausal women, we hypothesize that hepatic gluconeogenesis increases, thereby causing elevated blood glucose and hence increased HbA_{1c} levels over time. Prolonged hyperglycemia can cause oxidative stress in β cells.[35] E2 can prevent acute oxidative injury in β-cells in a hyperglycemic state by suppressing the β-cell translocation gene 2 (BTG_2)-p53-Bax pathway.[36] ERα localization in pancreatic β cells shows that E2 can confer protective effects against oxidative stress directly on β cells[37] and additionally in hepatocytes[38] to prevent insulin-deficient diabetes. A meta-analysis showed women undergoing HRT had alterations in metabolic syndrome components,[39] thereby supporting that perturbations in sex hormone levels can impair glucose homeostasis. These observations, together with mechanistic evidence, are consistent and support our results.

Strengths and limitations

To our knowledge, this study is the first population-based study to evaluate the relations between endogenous 17-OHP and glucose metabolism in both men and women. We have a relatively large sample size for the cross-sectional analyses from a well-characterized population-based study in men and women. This allowed us to adjust for numerous potential confounders. Another strength of this study is the prospective design with OGTT data available at both baseline and follow-up, allowing us to investigate not only the development of clinically diagnosed T2D but also of early derangements in glucose metabolism and newly OGTT-diagnosed T2D. However, this study also has limitations. While we adjusted our results for many established T2D risk factors, we did not have detailed dietary information, and the possibility of residual confounding cannot be precluded. Additionally, in the cross-sectional analyses, we cannot clearly distinguish cause and effect. Also, we could not identify women with polycystic ovarian syndrome (PCOS) in our dataset as the information is unavailable. PCOS symptoms persist even in postmenopausal women and could cause perturbations in sex hormone concentrations and, thus, metabolic processes. Lastly, we could not account for the effects of change in endogenous progestogens and estrogens, as the sex hormones were measured only at baseline.

CONCLUSIONS

Our findings support an inter-relation between endogenous female sex hormones and altered glycemic

metabolism not only in middle-aged and elderly women but also in men. However, future studies should corroborate our findings in both men and women, in well-powered settings, with sufficient follow-up, and investigate directional associations through Mendelian randomization.

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Contributors LHYL and BT designed the study. AC, TZ, CP, WR, JA, AP, and BT contributed data. LHYL performed all data analyses with guidance from FS and BT, and is the guarantor of this work. Result interpretation was done by LHYL, JN, and BT. LHYL wrote the manuscript with guidance from JN. and BT. All authors critically revised and approved the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The KORA F4 and FF4 studies were carried out following the Declaration of Helsinki, including written consent from all participants. All study methods were approved by the ethics committee of the Bavarian Chamber of Physicians (Ethical Approval Number 06068).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The data are subject to national data protection laws and restrictions were imposed by the ethics committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with Cooperative Health Research in the Region of Augsburg (KORA) via the online portal KORA.passt (https://epi.helmholtz-muenchen.de/). Please contact the corresponding author, Barbara Thorand, in case of further questions.

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Associations of endogenous androgens and sex hormone-binding globulin with kidney function and chronic kidney disease

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Introduction: The role of endogenous androgens in kidney function and disease has not been extensively explored in men and women.

Research design and methods: We analyzed data from the observational KORA F4 study and its follow-up examination KORA FF4 (median follow-up time 6.5 years) including 1293 men and 650 peri- and postmenopausal women, not using exogenous sex hormones. We examined the associations between endogenous androgens (testosterone [T], dihydrotestosterone [DHT], free T [fT], free DHT [fDHT], and T/DHT), with estimated glomerular filtration rate (eGFR) at baseline and follow-up, prevalent, and incident chronic kidney disease (CKD) adjusting for common CKD risk factors.

Results: At baseline, 73 men (5.7%) and 54 women (8.4%) had prevalent CKD. Cross-sectionally, no significant associations between androgens and kidney function were observed among men. In women, elevated T (β=-1.305, [95%

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CI -2.290; -0.320]) and fT (β=-1.423, [95% CI -2.449; -0.397]) were associated with lower eGFR. Prospectively, 81 men (8.8%) and 60 women (15.2%) developed incident CKD. In women, a reverse J-shaped associations was observed between DHT and incident CKD ($P_{non\text{-}linear}$=0.029), while higher fDHT was associated with lower incident CKD risk (odds ratio per 1 standard deviation=0.613, [95% CI 0.369; 0.971]. Among men, T/DHT (β=-0.819, [95% CI -1.413; -0.226]) and SHBG ($P_{non\text{-}linear}$=0.011) were associated with eGFR at follow-up but not with incident CKD. Some associations appeared to be modified by type 2 diabetes (T2D).

Conclusion: Suggestive associations are observed of androgens and SHBG with kidney impairment among men and women. However, larger well-phenotyped prospective studies are required to further elucidate the potential of androgens, SHBG, and T2D as modifiable risk factors for kidney function and CKD.

KEYWORDS

testosterone (T), dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), chronic kidney disease, type 2 diabetes, kidney function

1 Introduction

Age-related kidney function decline can lead to CKD (1, 2). CKD development is accelerated by increasing prevalence of its risk factors such as obesity, smoking, hypertension and pre-eminently, T2D (2). As a well-known independent risk factor for cardiovascular and all-cause mortality, CKD represents a burgeoning silent epidemic straining healthcare systems (3). As CKD progresses faster in men (2), more focus has recently been placed on understanding the role of androgens in CKD development.

Aberrant androgen levels are a characteristic manifestation of CKD. Hypogonadism (a condition characterized by low T levels) is prevalent among men with CKD. Among women with CKD, androgen profiles are unclear (4), although women with metabolic syndrome show elevated T levels (5). Epidemiological reports in this context are inconsistent. Some observational cross-sectional (6) and prospective studies (7) report reduced kidney function with lower T levels in men, while others showed no differences (8, 9). In women, an observational prospective (9) and a Mendelian randomization (MR) study (10) found no relationships between T and kidney function. Further, despite being implicated in cardiovascular disease (CVD) (11) and sodium reabsorption (12), the role of DHT in kidney function has been barely evaluated (9, 13).

While bound to sex hormone-binding globulin (SHBG), a protein involved in sex hormone transportation, androgens are inactive. Meanwhile, free (unbound) androgens exert their effects in target tissues through androgen receptor binding (14). SHBG was prospectively-associated with better kidney function (9) and causally associated with lower CKD risk among men, but not among women (15). Few studies (9, 15) investigated the association between SHBG and kidney function in women. Therefore, additional investigations are needed.

Diabetic kidney disease (DKD) comprises 30-50% of CKD cases (16). T2D, representing an additional disease burden, could influence the relationship between androgens and kidney function. While sex-specific differences in androgen levels are evident in T2D (5), it remains unclear whether the putative associations between androgens and kidney function differ between person with and without T2D.

Therefore, the present study aimed to evaluate cross-sectional and prospective associations between levels of endogenous androgens and SHBG with measures of kidney function, as estimated by eGFR, in men and peri-/postmenopausal women from the general population. In addition, we aimed to assess whether any putative associations between androgens and kidney function were modified by the presence of T2D at baseline.

2 Methods

2.1 Study population

Data were obtained from the Cooperative Health Research in the Region of Augsburg (KORA) baseline (F4) (2006-2008)

and follow-up (FF4) studies (2013-2014). Both studies are follow-up examinations of the KORA S4 study (1999-2001) conducted in Augsburg, Southern Germany, and two surrounding counties. The study design has been described previously in detail (17).

The KORA F4 study included 3080 participants aged between 32 and 81 years, of which 2161 participated in KORA FF4. Three participants who withdrew consent and 570 premenopausal women were excluded from the analyses. After further exclusions as described in Figure 1, the final sample for the cross-sectional analyses comprised 1943 participants (1293 men and 650 peri-/postmenopausal women), while the prospective analyses sample comprised 1349 participants (933 men and 416 peri-/postmenopausal women) for follow-up eGFR and 1294 participants for incident CKD after exclusion of 55 participants with prevalent CKD.

2.2 Kidney function

Glomerular filtration rates were estimated based on serum creatinine concentrations using the Chronic Kidney Disease Epidemiology Collaboration formula (18). In the main analysis, prevalent CKD was defined as an eGFR <60 ml/min/1.73m² at baseline. Incident CKD was defined as eGFR <60 ml/min/1.73m² at follow-up in participants without prevalent CKD at baseline. Serum creatinine was measured in fresh serum with a modified Jaffe test (KREA Flex, Dade Behring) in F4 and the first part of FF4, whilst the standard Jaffe method was used in the second part of FF4 (Roche Cobas 8000 instrument). All measurements were calibrated to IDMS standards.

2.3 Androgen and SHBG quantification

Serum samples at baseline were collected and stored at -80°C until the analysis of androgens and SHBG. T and DHT were quantified in serum using liquid chromatography-electrospray ionization-tandem mass spectrometry and the AbsoluteIDQ Stero17 Kit (BIOCRATES Life Sciences, Austria) (19). The calibration, imputation, and normalization of sex hormone measurements are described in detail in the Supplementary Methods and Materials 1. For T measurements the intra-assay CV was 10.3%, the lower limit of quantification (LLOQ) was 0.35 nmol/L, and the upper limit of quantification (ULOQ) was 34.7 nmol/L. For DHT respective values were as follows: intra-assay CV: 11.1%, LLOQ: 0.04 nmol/L, ULOQ: 10.2 nmol/L. Intra-assay CVs were calculated using the means from five quality

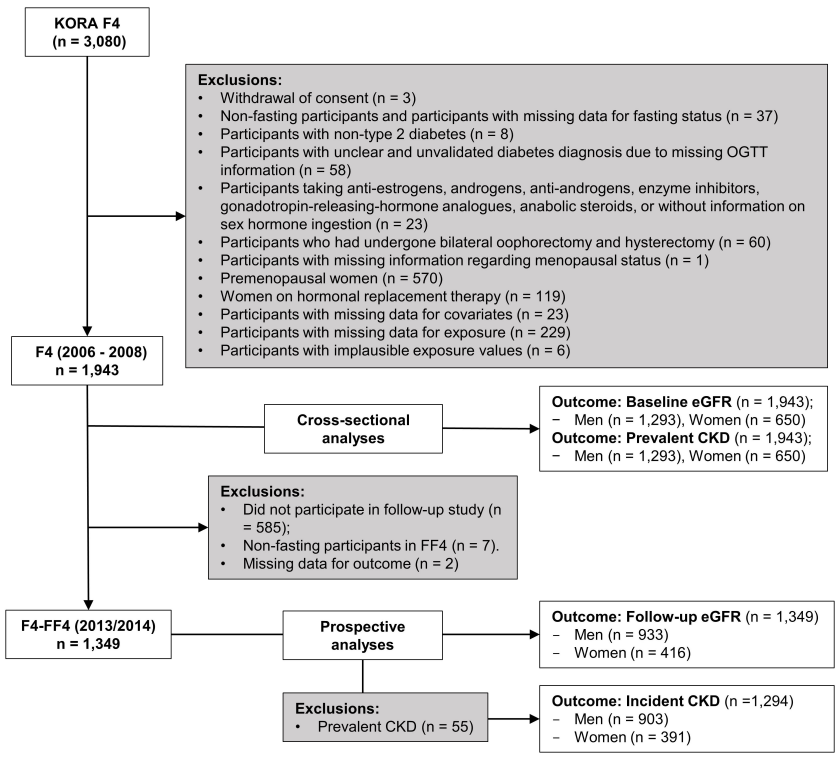

FIGURE 1
Exclusion flowchart.

control samples and means over thirty-nine plates. T/DHT was calculated by dividing T concentrations by those of DHT. fT and fDHT were calculated based on measured T, DHT, SHBG, and serum albumin using the formula derived by Mazer (20). SHBG in serum was quantified using the ARCHITECT SHBG assay, a chemilumineschent microparticle immunoassay (Abbott Laboratories, USA). SHBG samples had an intra-assay CV of 4.3%. Serum albumin was quantified using immunophelometry (ALB Flex; Dade Behring, Germany).

2.4 Covariates

At baseline, non-high-density lipoprotein cholesterol (non-HDL-C) was calculated by subtracting HDL-C from total cholesterol to account for all LDL cholesterol types. Total cholesterol and HDL-C were measured in fresh serum by enzymatic methods (CHOL Flex and AHDL Flex, Dade Behring). C reactive protein (CRP) was quantified from frozen plasma using a high-sensitivity latex-enhanced nephelometric assay (BN II Analyzer, Dade Behring). Thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), and free thyroxine (fT4) were quantified using immunochemiluminescent procedures (Dimension Vista System, Siemens, Germany).

Information on age, sex, waist circumference, prevalent cardiovascular diseases (CVD), anti-hypertensive medication usage, lipid-lowering medication usage, blood pressure, smoking status, alcohol consumption, and physical activity were assessed using a standardized interview, performed by trained medical staff (17). A participant was considered to have prevalent CVD if they had a history of either myocardial infarction, stroke, or angina pectoris. Participants' medication use within seven days before the examination was assessed by trained medical staff using the IDOM database (21). Smoking status was categorized as never smokers, former smokers, and current smokers (≥1 cigarette a day). Physical activity was estimated through two separate four-category interview questions regarding the time spent per week on sport activities in summer and winter. Possible answers were (1) >2 hours, (2) 1-2 hours, (3) <1 hour, and (4) none. Participants who had a total score of <5, obtained by summing the numbers (1)-(4) relating to winter and summer, were considered to be 'physically active'. Alcohol consumption was categorized into three groups: no consumption (0 g/day), moderate consumption (men 0.1-39.9 g/day, women 0.1-19.9 g/day), and high consumption (men ≥40 g/day, women ≥20 g/day) (22).

Previously known T2D was defined as a self-reported T2D diagnosis that was validated by a physician or medical chart review, or as self-reported current use of glucose-lowering mediation. Participants without known T2D obtained a standard 75g oral glucose tolerance test. Blood samples were taken without stasis after an overnight fast of ≥8 hours and 2 hours after glucose solution ingestion. Serum glucose was measured using hexokinase-G6PD (GLUFlex, Dade Behring, USA). Normoglycemia (NGT) (fasting glucose (FG) <6.1 mmol/L and 2 hour-glucose (2hG) <7.8 mmol/L), prediabetes [6.1 ≤ FG <7.0 mmol/L and 2hG <7.8 mmol/L (isolated impaired fasting glucose (IFG)] or FG <6.1 mmol/L and 7.8 ≤ 2hG <11.1 mmol/L (isolated impaired glucose tolerance (IGT)), or both (IFG and IGT)), and newly-diagnosed diabetes (FG ≥7.0 mmol/L or 2hG ≥11.1 mmol/L) were defined according to the 1999/2006 WHO criteria (23).

2.5 Statistical analyses

For baseline characteristics, categorical variables were presented as proportions (%), and continuous variables reported as mean (SD) or median (25^{th} and 75^{th} percentiles) for variables with normal and skewed distributions, respectively (Table 1). Natural log (ln)-transformations were applied to skewed variables to improve normality. Men and women were analyzed separately. Linear regression models were used to examine associations of baseline androgen and SHBG levels with continuous baseline and follow-up eGFR measures. Additionally, we examined the relationship between androgen and SHBG levels with prevalent and incident CKD using logistic regression. Exact time-to-event information regarding CKD manifestation during follow-up was unavailable, therefore logistic regression was used rather than survival analyses. Models with incident CKD as the outcome included men (n=903) and women (n=391) without prevalent CKD at baseline.

To enable sex-specific comparisons of association strengths across different sex hormones, effect estimates were calculated for a sex-specific one standard deviation (SD) increase in hormone concentrations. Models were adjusted for known risk factors for CKD: Age, waist circumference, systolic blood pressure, antihypertensive medication usage (yes/no), non-HDL-C, prevalent CVD (yes/no), lipid-lowering medication usage (yes/no), smoking status (never/former/current), physical activity (active/inactive), alcohol consumption (no/moderate/high), baseline diabetes status (NGT/prediabetes/T2D), and ln(CRP). Models with eGFR at follow-up and incident CKD as the outcome were additionally adjusted for baseline eGFR. Non-linearity was evaluated by including a non-linear term of hormone measurements in the models, and was visualized using restricted cubic splines. We evaluated the interaction between sex hormones and baseline diabetes status (i.e. NGT and prediabetes vs. T2D).

We performed additional sensitivity analyses: (1) We used a 3 SD cut-off for exclusion of participants with extreme androgen concentrations to ascertain the impact of extreme values on our estimates. (2) We further adjusted the models for TSH, fT3, and fT4 since thyroid hormones may impact androgen receptor expression and steroidogenesis (24, 25) and have been

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TABLE 1 Baseline characteristics.

	Men (n = 1293)	Women (n = 650)
Age (years)[a]	56 (13)	63 (9)
BMI (kg/m²)[a]	27.9 (4.2)	28.5 (5.3)
Waist circumference	98.5 (91.4, 106.1)	91.2 (82.5, 100.2)
Systolic BP (mmHg)[a]	128 (17.4)	120.8 (18.4)
Diastolic BP (mmHg) [a]	77.7 (10.1)	73.5 (9.4)
Antihypertensive medication (%)	411 (31.8)	265 (40.8)
Hypertension (%)	469 (36.3)	278 (42.8)
Prevalent cardiovascular diseases (%)	123 (9.5)	70 (10.8)
Total cholesterol (mmol/L)[a]	5.52 (0.99)	5.97 (1.02)
HDL-cholesterol (mmol/L)[a]	1.30 (0.32)	1.58 (0.37)
Non-HDL cholesterol (mmol/L)[a]	4.21 (0.98)	4.40 (1.00)
LDL-cholesterol (mmol/L)[a]	3.56 (0.86)	3.75 (0.93)
Triglycerides (nmol/L)[b]	1.33 (0.93, 1.94)	1.21 (0.87, 1.63)
C-reactive protein (mg/L)[b]	1.09 (0.55, 2.39)	1.50 (0.75, 3.06)
Lipid-lowering medication (%)	187 (14.4)	102 (15.7)
Smoking status (%)		
Never	393 (30.4)	374 (57.5)
Former	639 (49.4)	195 (30.0)
Current	261 (20.2)	81 (12.5)
Physically active (%)	709 (54.8)	357 (54.9)
Alcohol consumption (%)		
None	256 (19.8)	263 (40.5)
Moderate [c]	773 (59.8)	281 (43.2)
High [c]	264 (20.4)	106 (16.3)
Baseline diabetes status (%)		
Normal glucose tolerance	896 (69.3)	426 (65.8)
Prediabetes	232 (18.0)	137 (21.1)
Type 2 diabetes	165 (12.7)	85 (13.0)
Kidney function		
Baseline eGFR (ml/min/1.73m²) [a]	88.3 (16.2)	82.4 (15.7)
Follow-up eGFR (ml/min/1.73m²) [a]	81.0 (16.4)	75.6 (16.1)
eGFR change (ml/min/1.73m²/year) [b]	-1.18 (-2.22, -0.37)	-1.26 (-2.53, -0.41)
Baseline UACR (mg/g)	4.92 (3.14, 11.0)	7.23 (4.70, 12.9)
Follow-up UACR (mg/g)	4.12 (2.71, 8.30)	6.23 (4.08, 11.7)
Prevalent CKD [d] (%)	73 (5.7)	54 (8.4)
Incident CKD [e] (%)	81 (8.8)	60 (15.2)

(Continued)

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TABLE 1 Continued

	Men (n = 1293)	Women (n = 650)
Sex hormones		
Total T (nmol/L) [b]	14.6 (11.4, 18.6)	0.62 (0.42, 0.88)
Free T (pmol/L) [b]	191 (152, 228)	6.23 (4.20, 9.58)
DHT (nmol/L) [b]	1.25 (0.90, 1.71)	0.18 (0.10, 0.29)
Free DHT (pmol/L) [b]	6.85 (5.12, 8.81)	0.75 (0.41, 1.22)
T/DHT (unit) [b]	11.9 (9.47, 14.4)	3.47 (2.18, 5.97)
SHBG (nmol/L) [b]	48.2 (35.2, 65.4)	67.3 (48.3, 95.8)

[a]Measure of central tendency is presented as mean with corresponding standard deviation.
[b]Measure of central tendency is presented as median with corresponding 25th and 75th percentiles.
[c]Alcohol consumption defined as follows: moderate alcohol consumption (males 0.1-39.9 g/day and females 0.1-19.9 g/day), and high alcohol consumption (males ≥40 g/day and females ≥20 g/day).
[d]Defined as having an eGFR of <60ml/min/1.73m² at baseline.
[e]Defined as having an eGFR of <60 ml/min/1.73m² at follow-up. Those with prevalent CKD (n = 130; 73 males and 54 peri-/postmenopausal females) were excluded.
BMI, Body mass index; CKD, Chronic kidney disease; DHT, Dihydrotestosterone; eGFR, Estimated glomerular rate; HDL, High density lipoprotein; LDL, Low density lipoprotein; SHBG, Sex hormone-binding globulin; T, Testosterone; UACR, Urinary albumin to creatinine ratio.

associated with kidney function (26–28). (3) We adjusted further with T in SHBG models to ascertain the independency of SHBG on assessed outcomes. (4) We excluded perimenopausal women as sex hormone levels can fluctuate during this phase.

Significance levels were based on two-sided tests, where p-values of <0.05 were considered to be statistically significant. Statistical analyses were performed using R (v4.0.5).

3 Results

Baseline characteristics of the study participants are presented in Table 1. At baseline, 127 (6.5%) participants [73 (5.7%) men, 54 (8.4%) women] had prevalent CKD. During a median follow-up time of 6.5 (25^{th}, 75^{th} percentiles: 6.3, 6.6) years, 141 (7.2%) participants [81 (8.8%) men, 60 (15.2%) women] developed incident CKD. Due to the exclusion of premenopausal women in the present study, women were older than men; potentially explaining some baseline characteristic differences between men and women. Men had on average higher eGFR at baseline and follow-up, while women had steeper eGFR decline. As expected, total and free androgen levels as well as the ratio of T/DHT were considerably higher in men, while SHBG concentrations were higher in women.

In fully-adjusted models, among men, we did not observe any significant associations between T, DHT, and SHBG with baseline eGFR and prevalent CKD. Excluding extreme values (>3 SD) in a sensitivity analysis did not discernibly change these observations (Tables 2, S2, S4). Prospectively, higher T/DHT was associated with lower follow-up eGFR ($\beta_{T/DHT}$=-0.819, [-1.413; -0.226]). When extreme values were excluded, this association was attenuated to non-significance $\beta_{T/DHT}$=-1.181 [-2.685; 0.322] (Tables 3, S6). Also, a U-shaped association between baseline SHBG and follow-up eGFR was observed (β_{SHBG}=0.015, [-0.687; 0.717], β_{SHBG}^2 = 0.592, [0.133; 1.051]), $P_{non\text{-}linear}$=0.011) (Tables 3, S6, Figure 2A). Further adjustment with T did not discernibly change the results (β_{SHBG}=0.218, [-0.590; 1.026], β_{SHBG}^2 = 0.618, [0.156; 1.079]), $P_{non\text{-}linear}$=0.009). Among 933 men, 921 had fT3, fT4, and TSH measurements. This association remained significant after further adjustment for thyroid hormones (β_{SHBG}=0.051, [-0.655; 0.757], β_{SHBG}^2 = 0.696, [0.102; 1.037]), $P_{non\text{-}linear}$=0.017). Exclusion of extreme SHBG values (Table S6) did not discernibly alter this association. No significant associations between androgens, SHBG, and incident CKD were detected among men (Table S8).

Among women, elevated T and fT were inversely associated with baseline eGFR (β_T=-1.305, [-2.290; -0.320], β_{fT}=-1.423, [-2.449; -0.397]). When extreme T and fT values were excluded, these associations did not persist (β_T=-0.770, [-2.104; 0.565], β_{fT}=-0.721, [-2.117; 0.675]) (Tables 2, S3). Additionally, a non-linear association was observed between fDHT and prevalent CKD (β_{fDHT}=0.549, [0.324; 0.890], β_{fDHT}^2 = 1.151, [1.051; 1.291]), $P_{non\text{-}linear}$=0.007). Ultimately this non-linear association did not persist after excluding extreme fDHT values ($P_{non\text{-}linear}$=0.963) (Tables 2, S3). Instead, we observed a significant linear association (OR_{fDHT}=0.571, [0.328; 0.931]) (Table S5). A significant positive association between T/DHT and prevalent CKD ($OR_{T/DHT}$= 2.305, 1.069; 4.529) was observed only after excluding extreme values (Tables 2, S5). Prospectively, no significant associations were observed between androgens, SHBG, and follow-up eGFR among women (Tables 3, S7). A reverse J-shaped association was seen between DHT and incident CKD (β_{DHT}=0.579, [0.350; 0.938], β_{DHT}^2 = 1.230, [0.976; 1.450]), $P_{non\text{-}linear}$=0.025) (Table 3; Figure 2B). DHT values below the mean were inversely associated with incident CKD, while no association or possibly a very weak positive association was suggested for values

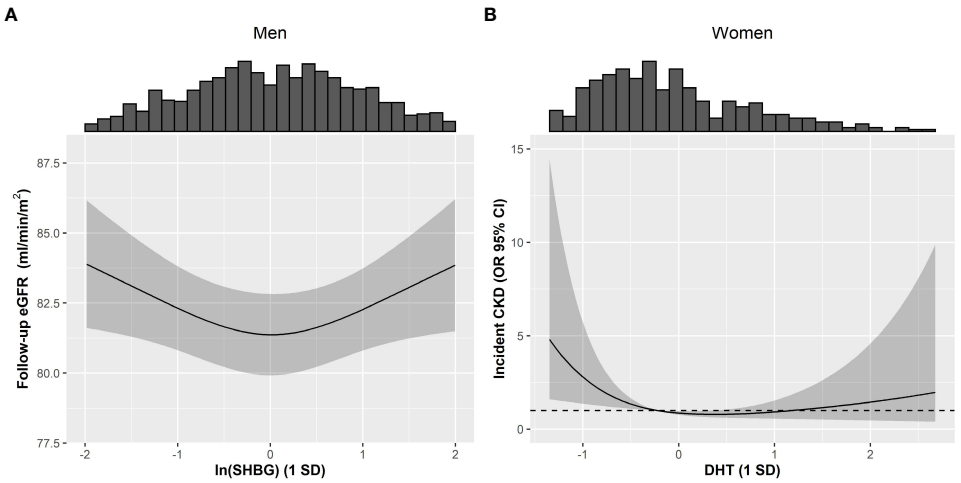

FIGURE 2
Restricted cubic splines describing non-linear associations between androgens or SHBG with kidney function in men and women of the KORA F4/FF4 study. Non-linear associations between **(A)** ln(SHBG) and follow-up eGFR in men, plus **(B)** between DHT and incident CKD in women. Non-linearity was investigated by introducing a quadratic term to the models for all exposures. Interaction between diabetes and DHT or SHBG was assessed using a 3-way interaction (i.e. DHT or SHBG*diabetes + (DHT or SHBG)^2*diabetes). Non-linear associations were not modified by diabetes ($P_{interaction}$: **(A)** 0.920 and **(B)** 0.948). Solid line represents the estimated spline function for follow-up eGFR and incident CKD, and the grey shaded area represents the respective 95% CI spline estimation. The histograms provide insight to the population density along ln(SHBG) and DHT values. Models were adjusted using Model 2, comprising of baseline eGFR, baseline age, waist circumference, systolic blood pressure, usage of antihypertensive medication, non-HDL cholesterol, prevalent cardiovascular disease, usage of lipid-lowering medication, smoking status, physical activity, alcohol consumption, diabetes status, and ln(CRP). CKD: Chronic kidney disease, CI: Confidence interval, CRP: C-reactive protein, DHT: Dihydrotestosterone, eGFR: Estimated glomerular filtration rate, HDL: High-density lipoprotein, OR: Odds ratio, SD: Standard deviation.

above the mean (Figure 2B). This association remained significant after exclusion of extreme DHT values (β_{DHT}=0.551, [0.334; 0.894], β_{DHT}^2 = 1.689, [1.156; 2.491]), $P_{non\text{-}linear}$=0.007) (Table S9). Among 391 women, 375 had fT3, fT4, and TSH measurements. The association between DHT and incident CKD did not persist after accounting for thyroid hormones (β_{DHT}=0.588, [0.343; 0.986], β_{DHT}^2 = 1.188, [0.868; 1.429]), $P_{non\text{-}linear}$=0.122). Additionally, an inverse association between fDHT and incident CKD was observed (OR_{fDHT}=0.613, [0.369; 0.971]) – which did not appreciably change after excluding extreme fDHT values (Tables 3, S9). Among 386 women, 370 women had fT3, fT4, and TSH measurements. The association between fDHT and incident CKD remained significant after further adjustment for thyroid hormones (OR_{fDHT}=0.613, [0.359; 0.993]). Notably, all observations among women did not significantly change when perimenopausal women were excluded.

Diabetes status was a significant effect modifier in several models. Among men, diabetes modified the association between T and follow-up eGFR ($P_{T\times diabetes}$=0.041). In men without diabetes, eGFR decreases ($\beta_{T(No\ diabetes)}$=-0.425, [-1.130; 0.279]), whereas in men with diabetes, eGFR increases ($\beta_{T(Diabetes)}$=1.709, [-0.679; 4.097]) as T levels increased. Among women, diabetes modified the association between T and baseline eGFR ($P_{T\times diabetes}$=0.014) and between DHT and prevalent CKD ($P_{DHT\times diabetes}$=0.001). Compared to women without diabetes, those with diabetes have steeper eGFR decline (β=-2.978, [-5.342; -0.614]) vs. β=-0.739, [-1.838; 0.361]) as T levels increased. However, due to the small sample size, we observed exceedingly wide CIs of the interaction term between DHT and diabetes status for prevalent CKD among women. Thus, stratified analysis was not performed.

4 Discussion

In the present study, we found several suggestive associations linking androgens and SHBG to kidney health among men and women. Among men, while no associations were observed between androgens, eGFR, and CKD, SHBG showed a U-shaped association with follow-up eGFR that was independent of T and not modified by diabetes status. In women, DHT showed a reverse J-shaped association with incident CKD, while elevated fDHT was significantly associated with lower CKD prevalence and incidence. Additionally, a higher T/DHT ratio was associated with higher CKD prevalence. Taken together, these findings suggest involvement of endogenous androgens and SHBG in CKD pathophysiology.

Our cross-sectional results in men regarding the lack of association of T and DHT with eGFR and CKD agree with the Diabetes Prevention Program Outcomes Study (DPPOS) that

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TABLE 2 Cross-sectional associations of endogenous androgens and SHBG with baseline eGFR and prevalent CKD.

Exposure [a]	Baseline eGFR β (95% CI)		Prevalent CKD OR (95% CI)	
	Men (n = 1,293)	Women (n = 650)	Men (n = 1,293)	Women (n = 650)
T	0.210 (-0.501 – 0.920)	**-1.305 (-2. 290 – -0.320)** [b]	0.810 (0. 597 – 1.081)	1.114 (0. 846 – 1.446)
fT	-0.034 (-0. 750 – 0.682)	**-1.423 (-2. 449 – -0.397)** [b]	0.765 (0.549 – 1.054)	1.166 (0. 874 – 1.539)
DHT	0.285 (-0. 395 – 0.965)	-0.197 (-1. 195 – 0.802)	0.964 (0. 663 – 1.327)	0.767 (0.484 – 1.132)
fDHT $fDHT^2$	0.065 (-0. 611 – 0.740)	-0.358 (-1. 364 – 0.648)	0.910 (0. 571 – 1.353)	**0.549 (0. 324 – 0.890)** **1.151 (1.051 – 1.291)** [b]
T/DHT	0.430 (-0. 227 – 1.086)	-0.405 (-1. 382 – 0.572)	0.790 (0. 486 – 1.098)	1.213 (0.976 – 1.473) [b]
ln(SHBG)	0.372 (-0. 364 – 1.107)	-0.076 (-1. 190 – 1.037)	1.071 (0. 774 – 1.484)	1.027 (0.715 – 1.478)

[a]Non-linearity was investigated by introducing a quadratic term to the models for all exposures. Here, only significant (P<0.05) terms are reported. Quadratic terms, which were significant, were presented in this table along with corresponding linear terms (i.e. fDHT and $fDHT^2$ with prevalent CKD in women).
[b]Number of participants after exclusion of androgen or SHBG measurements >3 SD above/below the mean; Men: T (n = 1,273), fT(n = 1270), DHT (n = 1,268), fDHT (n = 1,271), T/DHT (n = 1,279), and ln(SHBG) (n = 1,289). Women: T (n = 636), fT(n = 638), DHT (n = 637), fDHT (n = 642), T/DHT (n = 640), and ln(SHBG) (n = 648). Following estimates were revealed for baseline eGFR: β_T = -0. 770 (-2. 104 – 0.565), β_{fT} = -0.721 (-2. 117 – 0.675), OR_{fDHT} = 0. 571 (0.328 – 0.931), and for prevalent CKD: $OR_{T/DHT}$ = 2.305 (1. 069 - 4.529). β-estimates are per 1 sex-specific SD and adjusted using Model 2, comprising of baseline age, waist circumference, systolic blood pressure, usage of antihypertensive medication, non-HDL cholesterol, prevalent cardiovascular diseases, usage of lipid-lowering medication, smoking status, physical activity, alcohol consumption, diabetes status, ln(CRP). Interaction by diabetes status was assessed by entering the interaction as a multiplicative term (i.e. androgen or SHBG*diabetes) in Model 2. For models with a significant quadratic term (i.e. $fDHT^2$), interaction was assessed using a 3-way interaction (i.e. fDHT*diabetes + $fDHT^2$*diabetes). Prevalent CKD was defined as a baseline eGFR <60 ml/min/1.73m^2. Bold values indicate statistical significance ($p < 0.05$).
CKD, Chronic kidney disease; CI, Confidence interval; CRP, C-reactive protein; DHT, Dihydrotestosterone; eGFR, Estimated glomerular filtration rate; HDL, High-density lipoprotein; SD, Standard deviation; SHBG, Sex hormone-binding globulin; T, Testosterone; T/DHT, T to DHT ratio.

assessed the associations between endogenous androgens and kidney measures over 11 years in 2170 participants (889 men, 1281 women) (9). Similar observations were made in 1470 men from the Third National Health and Nutrition Examination Survey (8). The tendency towards an inverse association of T (and fT) with follow-up eGFR in the present study was concordant with a randomized controlled trial in 48 hypogonadal men, which showed that 3-month and 6-month T treatment lowered eGFR (29). In contrast to the above investigations, Kurita et al. (6) reported that elevated endogenous T was cross-sectionally associated with higher eGFR among Japanese men. Differing T levels among men attributed to genetic differences could explain this (30).

In the present study, women with extreme T and fT levels appear to have driven the inverse cross-sectional relationship with eGFR. The null association between T, fT, and baseline eGFR after excluding women with extreme T and fT levels is consistent to observations from Kim et al. (9). Hyperandrogenism is common among women with polycystic ovarian syndrome (PCOS) and T2D (5, 31). Higher BMI and waist circumference, as well as lower eGFR levels among women with extreme T and fT levels in the current study (data not shown) are consistent to previous reports of higher adiposity (32–34) and higher risk of PCOS-associated (35) and non-PCOS-associated kidney dysfunction (35–38). Androgen excess is associated with visceral fat accumulation (33, 39–41) and endothelial dysfunction (42–45), both of which can drive kidney dysfunction (46). This potentially explains our finding regarding the initial inverse association between T levels and eGFR; particularly for the extreme T and fT levels among women. Even though there is evidence that T may compromise kidney function in women (12, 47), the link between androgens and kidney function has not been extensively investigated. Thus, additional studies are required to better understand these associations among women.

We additionally observed during sensitivity analyses, that an elevated T/DHT ratio (higher T levels in regards to DHT) was associated with higher CKD prevalence among women, and that higher circulating levels of fDHT were associated with a lower prevalence and incidence of CKD. Considering that T and DHT levels, as well as T/DHT ratios are maintained at least partially by 5α-reductase (an enzyme responsible for converting T to DHT) (14), the possibility of sex-specific changes in the expression or activity of 5α-reductases during endocrine disorders merits further investigation.

The positive association between higher SHBG levels and eGFR at follow-up in men with SHBG levels above the mean, together with the tendency for an inverse association between SHBG and incident CKD was partially consistent to findings from an observational study (9) and an MR study that reported

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TABLE 3 Prospective associations between endogenous androgens and SHBG with follow-up eGFR and incident CKD.

Exposure [a]	Follow-up eGFR β (95% CI)		Incident CKD OR (95% CI)	
	Male (n = 933)	Female (n = 416)	Male (n = 903)	Female (n = 391)
T	-0.252 (-0.931 – 0.427)	-0.097 (-1.148 – 0.953)	0.851 (0.597 – 1.198)	0.932 (0.593 –1.432)
fT	-0.521 (-1.192 – 0.150)	-0.224 (-1.324 – 0.877)	0.875 (0. 594 – 1.272)	0.745 (0.429 – 1.231)
DHT DHT^2	0.476 (-0.169 – 1.122)	0.310 (-0.755 – 1.376)	0.731 (0. 452 – 1.150)	**0.579** **(0. 350 – 0.938)** **1.230** **(0. 976 - 1.450)** [c]
fDHT	0.156 (-0.486 – 0.797)	0.285 (-0.777 – 1.348)	0.828 (0. 475 – 1.394)	**0.613** **(0. 369 – 0.971)**
T/DHT	**-0.819** **(-1.413- -0.226)** [b]	-0.215 (-1.190 – 0.759)	1.290 (0.662 – 2.519)	1.559 (0.692 – 3.605)
ln(SHBG) $ln(SHBG)^2$	**0.015** **(-0. 687 – 0.717)** **0.592** **(0.133 - 1.051)**	0.558 (-0.620 – 1.736)	0.845 (0. 604 – 1.179)	1.207 (0.804 – 1.836)

[a]Non-linearity was investigated by introducing a quadratic term to the models for all exposures. Here, only significant quadratic terms (P<0.05) are reported. Quadratic terms which were significant were presented in this table along with corresponding linear terms (i.e. ln(SHBG) and $ln(SHBG)^2$ with follow-up eGFR in men, as well as DHT and DHT^2 with incident CKD in women). No other quadratic associations were observed in the main analyses.

[b]Number of participants after exclusion of sex hormone or SHBG measurements 3 SDs above/below the mean for follow-up eGFR; Men: T (n = 920), fT(n = 920), DHT (n = 913), fDHT (n = 915), T/DHT (n = 923), and ln(SHBG) (n = 931). Women: T (n = 408), fT(n = 410), DHT (n = 408), fDHT (n = 411), T/DHT (n = 410), and ln(SHBG) (n = 415). For incident CKD; Men: T (n = 890), fT(n = 891), DHT (n = 884), fDHT (n = 885), T/DHT (n = 893), and ln(SHBG) (n = 901). Women, T (n = 384), fT (n = 386), DHT (n = 383), fDHT (n = 386), T/DHT (n = 387), and ln(SHBG) (n = 390). After exclusion of measurements >3 SD, the association between T/DHT and follow-up eGFR in men was attenuated to non-significance (β = -1.181, [-2.685; 0.322]).

[c]Of 391 women, 375 had fT3, fT4, and TSH measurements. After adjusting for thyroid hormones, the non-linear association between DHT and incident CKD was attenuated to non-significance (β_{DHT}=0.588, [0.343; 0.986], β_{DHT^2} = 1.188, [0.868; 1.429]), $P_{non\text{-}linear}$=0.122)

ORs are per 1 sex-specific SD and adjusted using Model 2, comprising of baseline age, baseline eGFR, waist circumference, systolic blood pressure, usage of antihypertensive medication, non-HDL cholesterol, prevalent cardiovascular disease, usage of lipid-lowering medication, smoking status, physical activity, alcohol consumption, diabetes status, and ln(CRP). Interaction by diabetes status was assessed by entering the interaction as a multiplicative term (i.e. androgen/SHBG*diabetes) in Model 2. For models with a significant quadratic term (i.e. DHT^2 and $ln(SHBG)^2$), interaction was assessed using a 3-way interaction (i.e. DHT or ln(SHBG)*diabetes + DHT^2 or $ln(SHBG)^2$*diabetes). Incident CKD was defined as a follow-up eGFR <60 ml/min/1.73m^2 in participants without prevalent CKD.

CKD, Chronic kidney disease; CI, Confidence interval; CRP, C-reactive protein; DHT, Dihydrotestosterone; eGFR, Estimated glomerular filtration rate; HDL, High-density lipoprotein; SD, Standard deviation; SHBG, Sex hormone-binding globulin; T, Testosterone; T/DHT, T to DHT ratio.

significant associations between genetically-predicted higher SHBG concentrations and lower CKD risk, independent of T (15). In women, a report (9) regarding SHBG was also consistent to our results as no association between SHBG levels and kidney function were observed. Notably, the abovementioned MR study (15) also did not find an association between genetically-predicted higher SHBG and CKD risk among women. Despite limited studies providing mechanistic insights linking SHBG and CKD, inflammation and insulin resistance could mediate this link. An *in vitro* experiment showed that SHBG suppresses inflammation - an effect not altered by simultaneous T or E2 supplementation (48). Based on further MR reports, genetically-predicted higher SHBG may lower insulin resistance and T2D risk in men and women (49–51). Moreover, in men, elevated fasting insulin has been linked to impaired kidney function, but not vice versa (52). Further studies that can provide sex-specific mechanistic insights to the SHBG-kidney relationship are warranted.

In the present study, we noted that adjustment for lipid levels and prevalent CVD consistently attenuated measures of association. Some plausible mechanisms linking androgens to kidney impairment include mediation through CV risk factors such as inflammation, hypertension (53), and hyperlipidemia (54). In patients with CKD, these risk factors are highly prevalent and contribute to atherosclerotic vascular disease, accounting for a majority of lesions that cause blood flow disruption to renal arteries (55). T can worsen atherosclerosis (56, 57), through its proinflammatory effects on the vascular system (58, 59). Hence, progression to kidney failure is accelerated. However, RCTs that attempted to assess CV safety associated with testosterone replacement therapy (TRT) in hypogonadal men (60–62) and postmenopausal women (63–65), showed no conclusive evidence that T supplementation is associated with increased CV risk (66). Albeit, these findings until now are derived from underpowered trials. Evidence concerning DHT is sparse. The few trials assessing the effects of DHT supplementation usually focused on DHT effects on prostate (67–69), rather than CV effects. Nevertheless, exogenous DHT has been shown to lower total and LDL-cholesterol, indicating favorable effects of DHT on traditional CVD risk factors in men (69).

RCTs to date have not investigated kidney outcomes following reinstatement of physiological androgen levels *via* TRT. Additionally, investigations in women are still lacking. Thus, further large-scale RCTs or population-based studies should assess the effects of androgens on kidney health in both sexes.

In the present study, baseline eGFR increases with higher T levels among men with T2D. Although T (and fT) have been inversely associated with T2D risk in epidemiological studies in men (70), glomerular hyperfiltration has been observed during early CKD stages among individuals with T2D (71); potentially explaining the opposing effects seen between eGFR and T among men with and without T2D. Moreover, diabetes-associated tubular hyperplasia and hypertrophy, as well as proximal tubular hyper-reabsorption reduces pressure in Bowman's space. This perpetuates hyperfiltration (72). As shown in the current study population, women with T2D showed steeper eGFR decline compared to their counterparts without diabetes, and had higher T levels (5). As oxidative stress is more apparent in the hyperglycemic state (73), the cumulative effect of higher T levels and increased oxidative stress burden could accelerate the deterioration of kidney function. However, the link between sex hormones and DKD remains ambiguous and further investigations are needed.

To our knowledge, this is the first epidemiological study evaluating the role DHT in kidney function among women. Strengths of the current study include the prospective design, allowing the examination of prospective associations between endogenous androgens, SHBG, and changes in kidney function. As our study population was well-characterized, we were able to adjust for various relevant confounders. Further, mass spectrometry was used for androgen quantification. However, this study also has its limitations. Instead of a clinical CKD diagnosis, eGFR was used to define CKD. Sex hormone measurements were available only at baseline so we could not monitor the changes over time and evaluate their contribution to our outcomes of interest. However, single T measurements have been shown to be an adequate representation of the mean annual T levels (74). Next, we could not identify women with PCOS in our dataset as the information is unavailable. PCOS symptoms persist in postmenopausal women and could cause perturbations in sex hormone concentrations. Furthermore, we performed multiple comparisons as various associations between androgens, SHBG, and kidney function were examined. Due to the hypotheses-generating nature of our study, we did not adjust for multiple testing (75). Finally, despite adjusting for multiple risk factors for kidney disease, we cannot rule out any residual confounding or unmeasured confounders in the investigated associations.

Conclusion

The results of the present study suggest that androgens and SHBG could be markers of kidney function impairment within a general population. Specifically, in men, we observed a U-shaped association between SHBG and follow-up eGFR, whereas in women, those with lower DHT levels may have an increased CKD risk. Conditions that are associated with abnormal hormonal profiles, such as T2D could alter the link between androgens and kidney function. Larger well-phenotyped prospective studies are required to further elucidate the potential of androgens, SHBG, and T2D as modifiable risk factors for kidney function.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Data are available upon reasonable request. Data collection in the KORA study is done in cooperation with the University Hospital of Augsburg. The data are subject to national data protection laws and restrictions were imposed by the ethics committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with the Cooperative Health Research in the Region of Augsburg (KORA) *via* the online portal KORA.passt (https://helmholtz-muenchen.managed-otrs.com/external). Please contact the corresponding author, Barbara Thorand, in case of further questions. Requests to access these datasets should be directed to https://helmholtz-muenchen.managed-otrs.com/external.

Ethics statement

The studies involving human participants were reviewed and approved by Bavarian Chamber of Physicians (Ethical Approval Number 06068). The patients/participants provided their written informed consent to participate in this study.

Author contributions

LHYL and BT designed the study. CP, AC, TZ, WR, JA, AP, and BT contributed data. LHYL performed all data analyses with guidance from JN and BT, and is the guarantor of this work. Result interpretation was done by LHYL, JN, and BT. LHYL wrote the manuscript with guidance from JN and BT. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1000650/full#supplementary-material

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Appendix 1

Paper 3 - Associations of testosterone with mortality and health outcomes among adults undergoing hemodialysis: A prospective cohort study

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Prospective Study of Associations Between Testosterone, Mortality, and Health Outcomes Among Adults Undergoing Hemodialysis

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INTRODUCTION

Men with kidney failure and diabetes mellitus (DM) commonly have low testosterone (T) levels,[1] which have been associated with increased risk of all-cause mortality and cardiovascular (CV) events during hemodialysis (HD).[2] In addition, low T levels are associated with a higher risk of incident DM[3] and lower health-related quality of life,[4] which in turn is linked to an increased risk of CV disease and premature mortality compared to otherwise similar men on HD with normal T levels.[1]

Despite known diabetes-mortality associations,[4] the effects of DM on the relationship between T, mortality rates, and health-related quality of life among HD patients is understudied. Investigating whether diabetes modifies the association between low T, mortality, and poor health-related quality of life in male HD patients could enable early risk stratification and improve patient care management, given the potential for worsened CV outcomes through the intersection of diabetes, abnormal hormonal milieu, and vascular endothelial dysfunction.

Given the potential for increased mortality risk and adverse outcomes in men on HD with low T and DM, we extended our previous work[5] using data from a prospective, multicenter study to evaluate whether DM or age would influence prospective associations between T levels, mortality, and adverse clinical outcomes in a cohort of male HD patients.

RESULTS

This study was reported according to Strengthening the Reporting of Observational Studies in Epidemiology guidelines (Supplementary Material). The baseline characteristics of 587 men analyzed in this study are shown in Table 1 (Supplementary Methods, Supplementary References, Figure S1), with 317 (54%) having low T levels, 170 (29%) having borderline T levels, and 100 (17%) having normal T levels. Of those with low, borderline, and normal T levels, 54.4%, 47.1%, and 39.9% had DM, respectively.

Over a median follow-up of 3.7 years (interquartile range: 1.4–8.0; range: 5 days–13.1 years), 318 (54%) participants died, and 118 (37%) of those deaths were attributed to CV-related causes. There were no significant associations between low T and all-cause mortality (hazard ratio [HR] = 1.24, 95% confidence interval [CI]: 0.97, 1.59), or CV mortality (HR = 0.85, 95% CI: 0.57, 1.27) (Supplementary Table S1). There was no significant

Table 1. Baseline characteristics

Characteristic	Low testosterone ($n = 318$)	Non-low testosterone ($n = 269$)	*P*-value
Age (yrs)	64 (54, 73)	61 (48, 72)	0.009
BMI (kg/m^2)	27 (23, 31)	26 (23, 30)	0.116
Systolic BP (mm Hg)	135 (118, 151)	141 (127, 152)	0.002
Ethnicity (%)			
White	260 (81.8)	201 (74.7)	0.116
Indigenous	16 (5.0)	18 (6.7)	
Other	42 (13.2)	50 (18.6)	
Smoker (%)	56 (17.7)	61 (22.8)	0.125
GN/autoimmune (%)	54 (17.0)	62 (23.0)	0.066
Comorbidities (%)[a]			
Prevalent CVD	183 (57.5)	115 (42.8)	<0.001
Mental health	104 (32.7)	91 (33.8)	0.773
Other serious illnesses	107 (33.6)	89 (33.1)	0.885
Health-related quality of life			
HUI3	0.71 (0.51, 0.91)	0.84 (0.61, 0.93)	<0.001
KDQOL12-PCS	31 (25, 37)	35 (28, 42)	<0.001
KDQOL12-MCS	48 (36, 55)	47 (37, 55)	0.600
Albumin (g/l)	33 (29, 37)	35 (31, 38)	0.040
Sex hormone-binding globulin (nmol/l)	33 (23, 46)	47 (35, 62)	<0.001
Testosterone (nmol/l)	4.8 (3.1, 6.4)	11.1 (9.4, 13.7)	<0.001
Free testosterone (pmol/l)	89 (56, 121)	169 (137, 205)	<0.001

BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; GN, glomerulonephritis; KDQOL 12-MCS, Kidney Disease Quality of Life Mental Component Score; KDQOL 12-PCS, Kidney Disease Quality of Life Physical Component Score.
[a]Comorbidities include CVD (stroke, coronary artery disease, heart failure, peripheral vascular disease), mental health (substance misuse, psychiatric disorder), and other serious illnesses (cancer, chronic respiratory disease, chronic liver disease, dementia). Measure of central tendencies are reported as medians with corresponding 25th and 75th percentiles.

association between low T and incident CVD (HR = 0.87, 95% CI: 0.53, 1.43) (Figure 1). In sensitivity analyses, continuous serum total T was associated with increased incident CVD risk (HR = 1.31, 95% CI: 1.04, 1.64).

Low T was prospectively associated with lower HUI3 scores ($MD_{HUI3} = -0.06$, 95% CI: −0.10, −0.02) and KDQOL12-PCS scores ($MD_{KDQOL12\text{-}PCS} = -1.87$, 95% CI: −3.62, −0.11), but not with KDQOL12-MCS scores ($MD_{KDQOL12\text{-}MCS} = -0.01$, 95% CI: −1.98, 1.97) (Supplementary Table S2). The association between serum T levels and HUI3 varied between younger (<63 years) and older (≥63 years) men (interaction $P = 0.044$) with serum T levels positively associated with better HUI3 scores only among older men ($MD_{HUI3} = 0.04$, 95% CI: 0.01, 0.06) (Supplementary Table S2). DM was not a significant effect modifier for any relationships (all interactions $P \geq 0.170$).

BIC values for linear models were lower for all-cause and CV mortality models, but for incident CVD, the BIC value for the nonlinear model was lower than that of the linear model (BIC 858 vs. 859) (Supplementary Figure S2C).

DISCUSSION

These findings update a previous study conducted by Bello *et al.*[5] using data collected over approximately 7 years from a prospective cohort of men undergoing HD in Canada. In this study, over a period of approximately 14 years, no significant associations were observed between low T, all-cause and CV mortality, and incident CV events. However, higher T levels may increase the risk of incident nonfatal CVD events. Furthermore, low T was significantly associated with lower HUI3 and KDQOL12-PCS scores, but not with KDQOL12-MCS scores. These associations were not modified by diabetes status or age, highlighting the potential of T as a modifiable risk factor across these HD patient subgroups.

Although not statistically significant, our findings on the association between low T levels and all-cause and CV mortality align with recent reports.[2,6] Men with lower T levels had a higher risk of all-cause mortality when T was examined as a continuous variable in a flexible model (Supplementary Figure S1A). On another note, sex-hormone-binding globulin determines T and fT concentrations, and higher sex-hormone-binding globulin levels has been associated with higher risk of death or CV event among men on HD.[6] Nilsson *et al.*[6] indicated that protein-energy wasting could explain the link between fT and outcomes during HD, but further investigation is warranted.

Notably, the directionality of our estimates is inconsistent with prior observational reports of associations between low T and higher risk of incident CVD. Incident CVD in prior reports was defined as a composite outcome comprising fatal and nonfatal CV events,[2] but we included only incident CVD in our definition. Therefore, different CV outcome definitions could explain the discrepancies. In addition, our data suggest that for every 1 SD increase in serum T, the risk of incident CVD increases. Our observations align with results of a meta-analysis, showing that T supplementation increases the risk of a CV-related event for older men.[7] Thus, more studies are needed to replicate our findings.

DM did not modify relationships between low T, mortality, and adverse health outcomes. The concomitant presence of low T and DM may worsen the inflammatory burden of HD patients. However, the moderate decrease in T levels among patients with diabetes similar to the decrease observed in chronic illnesses such as kidney failure suggest that low T could be a nonspecific biomarker of ill health.[8]

The positive association between serum total T and HUI3 scores were seen only in older patients (≥63 years), possibly because of healthy user bias. The relationship between low HUI3 scores and low T levels in our data suggest clinically-important changes,[9] but contextualizing minimal clinically-important scores

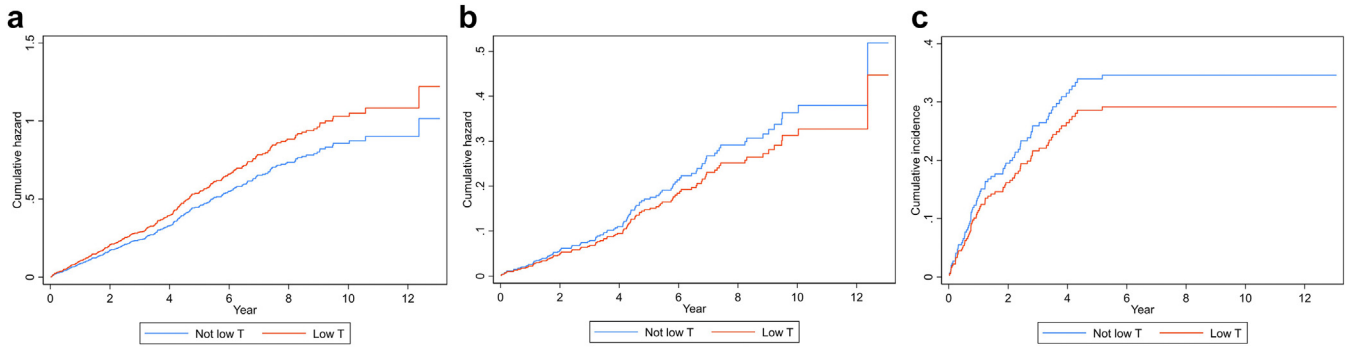

Figure 1. Cumulative hazard and incidence curves for the association between low T, all-cause mortality, CV mortality, and incident CVD. Cumulative hazard curves of low T vs. non-low T for (a) all-cause mortality and (b) CV mortality. Figure 1c represents the cumulative incidence curve of low T vs. non-low T for incident CVD. Figure 1a: Adjusted all-cause mortality HR = 1.24 (95% CI: 0.97, 1.59). Figure 1b: Adjusted CV mortality HR = 0.85 (95% CI: 0.57, 1.27). Figure 1c: Adjusted incident CVD HR = 0.87 (95% CI: 0.53, 1.43). HRs were adjusted for age, BMI, systolic blood pressure, ethnicity (white/indigenous/other), glomerulonephritis, DM, CVD (stroke, coronary artery disease, heart failure, peripheral vascular disease), smoking status (yes/no), mental health (substance misuse, psychiatric disorder), other serious illnesses (cancer, chronic respiratory disease, chronic liver disease, dementia), and SHBG. CI, confidence interval; CV, cardiovascular; CVD, cardiovascular disease; DM, diabetes mellitus, HR, hazard ratio; SHBG, sex-hormone-binding globulin; T, testosterone.

remains challenging in patients with kidney disease. Therefore, more research is needed in this area.

This study's strengths include data from a carefully-phenotyped HD population and a prospective (approximately 14 years), multicenter design. We were able to ascertain which participants were taking exogenous sex hormones, adjust for multiple confounders, such as malignancies and chronic respiratory disorders, and include data for health-related quality of life. Finally, serum T concentrations were measured using standardized methods in a well-established laboratory.

This study's limitations include a single T measurement at baseline, which did not allow monitoring of changes in T levels over time. Although the study tried to adjust for relevant confounders, residual confounding remains likely because of the nature of observational studies. We excluded participants with missing T measurements, but the findings were not likely impacted (Supplementary Table S3). Our findings are not generalizable because data used were from HD patients.

In conclusion, no significant associations were seen between low serum T levels and mortality (all-cause and CV). However, low T was associated with lower HUI3 and KDQOL12-PCS scores; and the positive association between serum total T and HUI3 scores were apparent only among older patients. Future work is needed to explore the impact of DM on serum T levels and the relationship to adverse health outcomes in patients on HD.

DISCLOSURE

PStenvinkel receives research support from Bayer for conducting a randomized trial on testosterone supplementation in male hemodialysis patients. All the other authors declared no competing interests.

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Data Availability Statement

The data are not publicly available because of national data protection laws and restrictions imposed by relevant research ethics boards to ensure data privacy of the

study participants. However, the data that support the findings of this study are available on reasonable request from the CKDCS Research Manager, Natasha Wiebe.

AUTHOR CONTRIBUTIONS

LL and AB developed the research idea and study design. LL and NW developed the statistical analysis plan. LL wrote the manuscript with support from NW and AB. NW, SR, SA, SWK, JJC, PStenvinkel, MT, AB acquired the data. NW analyzed the data and its interpretation were supported by LL, NW, SR, SA, SWK, JJC, PStenvinkel, PSenior, MT, AB, BT. AB is the guarantor of this work and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors provided critical feedback, helped shape the manuscript, and approved the final version of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Figure S1. Patient flowchart.

Figure S2. Nonlinear associations between testosterone, all-cause mortality, CV mortality, and incident CVD in men undergoing HD.

Table S1. Adjusted hazard ratios of all-cause mortality, CV mortality, and incident CV events.

Table S2. Adjusted mean differences for health-related quality of life measures.

Table S3. Baseline characteristics for excluded and included patients.

STROBE Checklist.

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Appendix 2

Paper 4 - Associations of estradiol with mortality and health outcomes among adults undergoing hemodialysis: A prospective cohort study

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Associations of Estradiol With Mortality and Health Outcomes in Patients Undergoing Hemodialysis: A Prospective Cohort Study

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Abstract

Background: Both lower and higher estradiol (E2) levels have been associated with increased mortality among women with kidney failure. However, robust data are still lacking.
Objective: We investigated the interaction of diabetes and age on linear and nonlinear associations between E2 levels, adverse outcomes, and health-related quality of life (HRQOL) in Canadian women undergoing hemodialysis (HD).
Design: Population-based cohort study; data from Canadian Kidney Disease Cohort Study (CKDCS).
Setting & patients: A total of 427 women undergoing HD enrolled in the CKDCS.
Measurements: Baseline E2 (in pmol/L) and E2 tertiles (<38 pmol/L, 38-95 pmol/L, >95 pmol/L).
Methods: Cox-proportional hazards used for all-cause and cardiovascular disease (CVD) mortality. Fine-Gray models used for incident CVD. Mixed models used for Health Utilities Index Mark 3 (HUI3), Kidney Disease Quality of Life Physical Component Scores (KDQOL12-PCS), and Mental Component Scores (KDQOL12-MCS).
Results: Over a median follow-up of 3.6 (interquartile range [IQR]: 1.6-7.5) years, 250 (58.6%) participants died; 74 deaths (29.6%) were CV-related. Among 234 participants without prior CV events, 80 (34.2%) had an incident CVD event. There were no significant linear associations between E2 and all-cause mortality, CVD mortality, and incident CVD. However, E2 showed a significant concave association with all-cause mortality, but not with CVD mortality and incident CVD. Among patients aged ≥63 years, higher E2 levels were associated with lower HUI3 scores, mean difference (MD) = –0.062 per 1 – SD pmol/L, 95% confidence interval (CI) = –0.112 to –0.012, but the opposite was observed in younger patients (<63 years) in whom higher E2 levels were associated with higher HUI3 scores (MD = 0.032 per 1 – SD pmol/L, 95% CI = 0.008-0.055), $P_{interaction}$ = .045. No associations were observed among E2, KDQOL12-PCS (MD = –0.15 per 1 – SD pmol/L, 95% CI = –1.15 to 0.86), and KDQOL12-MCS (MD = –0.63 per 1 – SD pmol/L, 95% CI = –1.82 to 0.57).
Limitations: Unmeasured confounding and small sample size.
Conclusions: The association between E2 and all-cause mortality may be nonlinear, while no association was observed for CVD mortality, incident CVD, KDQOL12-PCS, and KDQOL12-MCS. Furthermore, the association between serum E2 and HUI3 was modified by age: Higher levels were associated with higher utility among women aged <63 years and the converse observed among older women.

Abrégé

Contexte: Les taux faibles comme les taux élevés d'estradiol (E2) ont été associés à une mortalité accrue chez les femmes souffrant d'insuffisance rénale. Les données fiables à ce sujet font cependant encore défaut.
Objectif: Nous avons étudié l'incidence du diabète et de l'âge sur les associations linéaires et non linéaires entre les niveaux d'E2, les issues défavorables et la qualité de vie liée à la santé (QVLS) chez les Canadiennes suivant des traitements d'hémodialyse (HD).
Conception: Étude de cohorte en population réalisée à partir des données de la Canadian Kidney Disease Cohort Study (CKDCS).
Sujets et cadre de l'étude: 427 femmes sous HD inscrites à la CKDCS.
Mesures: Le taux d'E2 initial (pmol/L) et les taux d'E2 tertiles (<38 pmol/L; 38-95 pmol/L; >95 pmol/L).

Méthodologie: Des modèles à risques proportionnels de Cox ont été utilisés pour mesurer la mortalité toutes causes confondues et la mortalité liée aux maladies cardiovasculaires (MCV). Des modèles Fine-Gray ont été utilisés pour mesurer les MCV incidentes; et des modèles mixtes ont été utilisés pour calculer l'indice Health Utilities Index Mark 3 (HUI3) et les scores des composantes physique (KDQOL12-PCS [Physical Component Score]) et mentale (KDQOL12-MCS [Mental Component Score])) du questionnaire sur la qualité de vie (KDQOL).
Résultats: Au cours d'un suivi médian de 3,6 ans (intervalle interquartile [IIQ]: 1,6 à 7,5 ans), 250 participantes (58,6 %) sont décédées; 74 décès (29,6 %) étaient liés à un événement CV. Parmi les 234 participantes sans événements cardiovasculaires antérieurs, 80 (34,2 %) ont vécu un événement incident de MCV. Aucune association linéaire significative n'a été observée entre le taux d'E2 et la mortalité toutes causes confondues, la mortalité par MCV ou les MCV incidentes. Le taux d'E2 a cependant montré une association concave significative avec la mortalité toutes causes confondues, mais pas avec la mortalité par MCV ni avec les MCV incidentes. Chez les patientes âgées de 63 ans et plus, des taux élevés d'E2 ont été associés à des scores HUI3 plus faibles (différence moyenne [DM] = -0,062 par 1-SD pmol/L; intervalle de confiance à 95 % [IC95]: -0,112 à 0,012); alors qu'on a observé le contraire chez les patientes plus jeunes (< 63 ans), où des taux élevés d'E2 étaient plutôt associés à des scores plus élevés d'HUI3 (DM = 0,032 par 1-SD pmol/L; IC95: 0,008 à 0,055; *p*=0,045). Aucune association n'a été observée entre le taux d'E2, le KDQOL12-PCS (DM = -0,15 par 1-SD pmol/L; IC 95: -1,15 à 0,86) et le KDQOL12-MCS (DM = -0,63 par 1-SD pmol/L; IC95: -1,82 à 0,57).
Limites: Facteurs de confusion non mesurés; échantillon de petite taille.
Conclusion: Il pourrait exister une association non linéaire entre le taux d'E2 et la mortalité toutes causes confondues. Aucune association n'a toutefois été observée entre le taux d'E2 et la mortalité par MCV, les MCV incidentes, le KDQOL12-PCS et le KDQOL12-MCS. En outre, l'association entre le taux sérique d'E2 et l'HUI3 a été modifiée par l'âge: des taux plus élevés d'E2 ont été associés à un indice de santé plus élevé (HUI) chez les femmes âgées de moins de 63 ans, alors que l'inverse a été observé chez les femmes plus âgées.

Keywords
estradiol, hemodialysis, diabetes, all-cause mortality, cardiovascular mortality, health-related quality of life

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Introduction

Women with kidney failure are more likely to die prematurely compared with men with kidney failure—losing on average 3.6 years more life than men.[1] In patients with kidney failure, hypothalamic-pituitary-gonadal (HPG) axis disruption is common.[2] Elevated prolactin and disrupted gonadotropin-releasing hormone production results in lower estradiol (E2) production among women with kidney failure.[3] Due to the loss of cardioprotective and anti-inflammatory properties of E2, premature menopause, osteoporosis, and accelerated progression of cardiovascular disease (CVD) and mortality can occur as a result.[4]

Although most women with kidney failure are postmenopausal (older), their premenopausal counterparts exhibit E2 levels at postmenopausal levels.[5] Consequently, premature menopause and sexual dysfunction occur—2 conditions linked with increased mortality and CVD risks, as well as lower health-related quality of life (HRQOL) among women with kidney failure.[6-10] Chronic reduction in endogenous estrogen exposure (EEE) has been thought to contribute to increased mortality.[11,12]

Diabetes is common among patients with kidney failure.[9,13] Dialysis complicates glycemic control[14] and in turn increases CV risks and complications.[15] Patients with diabetes have higher mortality rates and worse health outcomes compared with those without diabetes.[16,17] Furthermore, mortality rates are higher in women with diabetes compared with their male counterparts.[7,17,18] However, uncertainty remains in regard to whether diabetes could alter associations among E2, mortality, and HRQOL in patients undergoing hemodialysis (HD).

Observational data have linked both low and high endogenous E2 levels with all-cause and CVD mortality[9] in women undergoing HD. We previously reported partially concordant results, where higher E2 levels were associated with increased all-cause mortality, but not with CVD mortality.[13]

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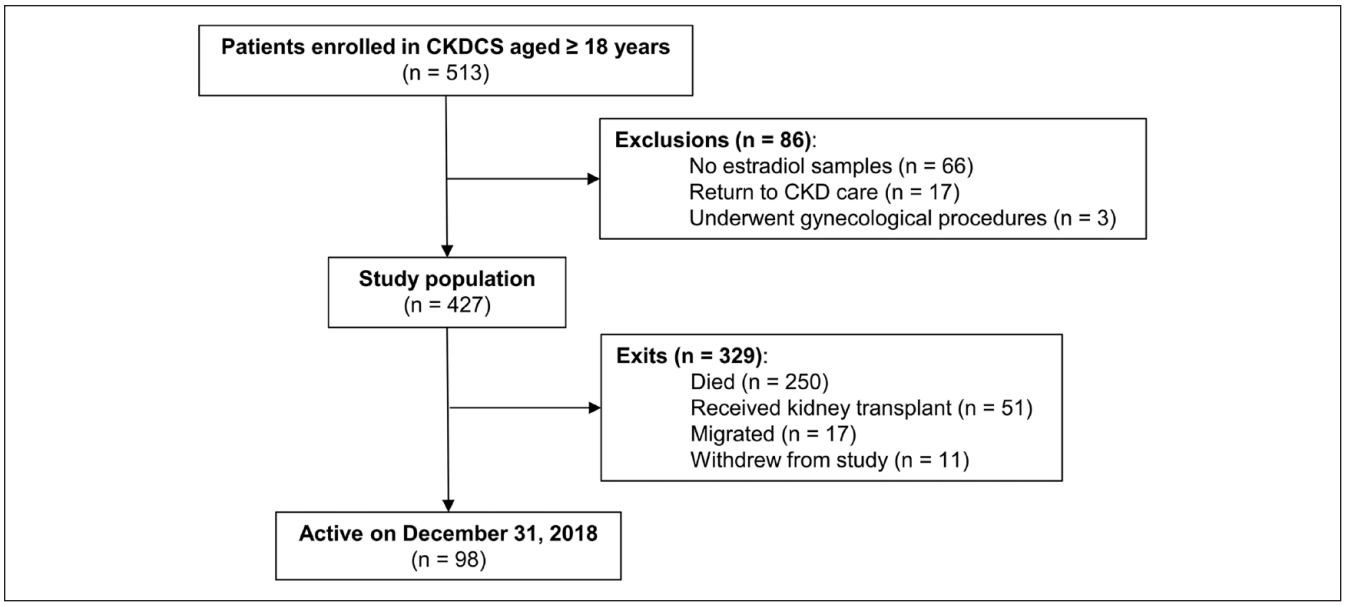

Figure 1. Participant flowchart.
Note. CKDCS = Canadian Kidney Disease Cohort Study; CKD = chronic kidney disease.

However, these observations were not as expected: In the general population, chronic E2 deprivation (eg, premature menopause) is associated with increased mortality and lower HRQOL.[2,4,19] In addition, despite the potential benefits of hormone replacement therapy (HRT) on HRQOL,[20] associations between endogenous E2 and HRQOL have not been extensively evaluated among women on HD. As Tanrisev et al[9] previously suggested possible nonlinear associations among E2, all-cause mortality, and CVD mortality, we aim to re-analyze the data using splines, also adding information on HRQOL.

E2 could be of prognostic and therapeutic relevance as high mortality and lower HRQOL associated with both HD and diabetes necessitate the identification of novel treatments to minimize those risks in kidney failure.[12,13] Using data from a prospective, multicenter study of women undergoing HD in Canada,[6] we investigated the linear and nonlinear associations among endogenous E2 levels, all-cause and CVD mortality, incident CVD, and HRQOL. We further investigated whether these associations are modified by diabetes or age, both of which could influence E2 levels and/or the risk of adverse outcomes.

Design and Methods

Study Design

We performed a secondary analysis of a prospective cohort study involving HD patients. Data were collected via participant interviews, chart reviews, and clinical databases at baseline (start of HD), month 6, and years 1, 2, 5, and 10. Demographics, medical and social history, weight, comorbidities, and HRQOL were ascertained at baseline and updated at each visit when participants received HD treatment. Modality transitions (ie, changes in dialysis types) were tracked throughout follow-up.[21]

Participants

Eligible participants were recruited between February 2005 and November 2012 from Alberta Kidney Care–North and South programs.[21] Written informed consent was obtained and relevant research ethics boards approved the study (Pro00002385, REB15-1048). This study is reported according to the STROBE guidelines.[22] Women (≥18 years old) initiating thrice weekly in-center HD across 4 Canadian dialysis centers (Calgary, Edmonton, Ottawa, and Vancouver) were eligible for inclusion. Participants who were unwilling or unable to provide informed consent, were without E2 measurements (n = 66), underwent gynecological procedures (n = 3), or returned to chronic kidney disease (CKD) care (n = 17) were excluded (Figure 1). In the current analysis, participants were followed until death (n = 250), kidney transplantation (n = 51), migration outside the study region (n = 17), withdrawal of consent (n = 11), or the end of the study period (December 31, 2018; n = 98), whichever came sooner. Details of the Canadian Kidney Disease Cohort Study are presented elsewhere.[21]

Covariates

Participants did a structured interview to collect information on demographic variables (age, sex, and ethnicity). Further parameters (ie, body mass index [BMI], predialysis systolic

blood pressure [SBP], albumin, diabetes, primary cause of kidney failure, smoking status, and comorbidities) were assessed by chart review. Smoking status was classified as nonsmokers (never smoked) or smokers (former and current smokers). Comorbidities included coronary artery disease, heart failure, hypertension, peripheral vascular disease, stroke, diabetes mellitus, chronic respiratory disease, cancers, chronic liver diseases, psychiatric illness, and substance misuse. None of the women included in the current study were taking exogenous sex hormones.

Estradiol

Sera from blood samples were collected at baseline within 3 months of HD session initiation. Sera were processed and frozen in 0.5 mL cryovials at –85°C within 72 hours of sample collection. Frozen sera samples were analyzed for baseline serum E2 at a central laboratory using certified routine methods (mass-spectrometry). Due to the high prevalence of amenorrhea among women with kidney failure,[23] menstrual status was not considered during sample collection. Ramesh et al[13] did not find significant associations between storage duration and HD center with E2 levels.

Outcomes

Primary outcomes were all-cause mortality, CVD mortality, and incident CVD (stroke, transient ischemic attack, coronary artery disease, heart failure, or peripheral vascular disease). Death was ascertained by chart review, and other outcomes were determined based on administrative data from the provincial health ministry (ie, Alberta Health). Our algorithm for defining CVD mortality with *International Classification of Diseases, Tenth Revision* (*ICD-10*) codes has been published previously.[24] Incident CVD was captured for those without prevalent CVD at baseline. Secondary outcomes were HRQOL, assessed using the Health Utilities Index Mark 3 (HUI3) instruments[25] and the physical (PCS) and mental (MCS) component scores of the Kidney Disease Quality of Life (KDQOL12) instrument.[26] HUI3 scores range from –0.36 to 1.00; scores below 0 reflects a health state that was considered to be worse than death, whereas 1.00 indicates perfect health. The suggested minimal clinically important difference (MCID) for HUI3 scores is 0.03.[25,27,28] KDQOL12-PCS and KDQOL12-MCS values range from 0 to 100, where 0 indicates death and 100 indicates perfect health.

Statistical Analyses

Descriptive statistics of the study participants are presented stratified by tertiles of E2, and shown as frequency (percentages) for categorical variables or medians (interquartile range [IQR]) for continuous variables. E2 was analyzed as a continuous variable to increase statistical power. The association among E2, all-cause mortality, and CVD mortality was estimated by calculating hazard ratios (HRs) and 95% confidence intervals (CIs) using Cox-proportional hazards models. The association among E2 and incident CVD, HRs, and their corresponding 95% CIs were calculated using Fine-Gray models (subdistribution hazard) with death as a competing risk.[29] For the prospective associations of E2 with HRQOL measures (ie, HUI3, KDQOL12-PCS, and KDQOL12-MCS scores), difference in means (MDs) and their corresponding 95% CIs were calculated using linear mixed models. Participants were modeled as random effects and visit timepoints (ie, baseline, month 6, years 1, 2, 5, and 10) as fixed effects.

As Tanrisev et al[9] previously suggested possible nonlinear associations among E2, all-cause mortality, and CV mortality, we parametrized E2 with restricted cubic splines with 3 knots (placed at the fifth, 50th, and 95th percentiles). As the splines indicated a concave shape, we replaced the restricted cubic splines with quadratic and linear terms for E2. Nonlinearity was tested with this latter model.

All models were adjusted for baseline age, BMI, ethnicity, predialysis SBP, glomerulonephritis, diabetes, CVD, mental health (smoking status, substance misuse, and psychiatric disorder), other serious illnesses (cancer, chronic respiratory disease, chronic liver disease, and dementia), and albumin. Linear and quadratic terms were used for BMI, given its U-shaped association with mortality. Missing data were present for the following covariates: BMI (1.6%), predialysis SBP (1.2%), and serum albumin (11.0%). Thus, missing data were estimated via multiple imputations using multivariable normal regression; the number of iterations (16) was greater than the maximum fraction (11%) of missingness.[30] In subgroup analyses, we assessed whether diabetes status (yes vs no) and age (above or below the median age of 63 years) modified the associations between E2 and assessed outcomes using interaction terms.

We analyzed E2 as tertiles during sensitivity analysis due to skewness of E2 measurements and the limited interpretability of log-transforming the exposure variable. A 2-sided p value of <0.05 was used as a threshold for statistical significance. All analyses were done in Stata/MP 17.0 (www.stata.com).

Results

Baseline characteristics of 427 women undergoing HD stratified by tertiles are shown in Table 1. Median follow-up was 3.6 years (IQR: 1.6–7.5 years; range: 4 days–13.7 years). The median age of participants was 63 years (50–73 years) and the majority were white (76.1%). Most women had diabetes (53.6%). Of 229 patients with diabetes, 21 (9.2%) had type 1 diabetes. Women with the highest E2 levels were younger, had lower BMI, lower systolic blood pressure, and were more likely to smoke. While women in the middle tertile had the lowest HUI3 and KDQOL12-PCS scores, women

Table 1. Baseline Characteristics of the Study Population.

Characteristic	Low E2 tertile (<38 pmol/L) (n = 140)	Middle E2 tertile (38-95 pmol/L) (n = 144)	High E2 tertile (>95 pmol/L) (n = 143)	*P* value
Age (years)	64 (57, 75)	67 (57, 74)	56 (41, 66)	<.001
BMI (kg/m^2)	27 (23, 31)	27 (22, 33)	26 (21, 34)	.939
Systolic BP (mmHg)	141 (125, 154)	138 (124, 158)	135 (115, 151)	.021
Ethnicity (%)				
White	106 (75.7)	114 (79.2)	105 (73.4)	.169
Indigenous	10 (7.1)	16 (11.1)	20 (14.0)	
Other	24 (17.1)	14 (9.7)	18 (12.6)	
Smoker (%)	14 (10.0)	23 (16.0)	29 (20.4)	.053
Diabetes (%)	73 (52.1)	80 (55.6)	76 (53.1)	.838
GN/autoimmune (%)	17 (12.1)	25 (17.4)	29 (20.3)	.177
Comorbidities[a] (%)				
CVD	63 (45.0)	72 (50.0)	58 (40.6)	.275
Mental health	42 (30.0)	46 (31.9)	55 (38.5)	.286
Other serious illnesses	47 (33.6)	43 (29.9)	33 (23.1)	.141
HRQOL measures				
HUI3	0.72 (0.46, 0.85)	0.64 (0.42, 0.83)	0.69 (0.48, 0.87)	.536
KDQOL12-PCS	31 (26, 38)	30 (24, 39)	31 (25, 37)	.900
KDQOL12-MCS	48 (38, 58)	43 (37, 54)	43 (35, 50)	.081
Albumin (g/L)	34 (31, 37)	34 (31,37)	32 (28, 36)	.053
Estradiol (pmol/L)	19 (19, 29)	63 (48, 77)	169 (118, 294)	<.001

Note. Measure of central tendencies is reported as medians with corresponding 25th and 75th percentiles. *P* values calculated for differences only between estradiol tertiles. E2 = estradiol; BMI = body mass index; BP = blood pressure; CVD = cardiovascular disease; GN = glomerulonephritis; HRQOL = health-related quality of life; HUI3 = Health Utilities Index mark 3 scores; KDQOL12-PCS = Kidney Disease Quality of Life Physical Component Score; KDQOL12-MCS = Kidney Disease Quality of Life Mental Component Score.

[a]Comorbidities include CVD (cerebrovascular disease, coronary artery disease, heart failure, and peripheral vascular disease), mental health (smoking status, substance misuse, and psychiatric disorder), and other serious illnesses (cancer, chronic respiratory disease, chronic liver disease, and dementia).

in the lower tertile had higher KDQOL12-MCS scores. Moreover, median E2 levels did not differ based on diabetes status (63 vs 65 pmol/L; $P = .750$).

Over the study period, 250 (58.6%) participants died, and 74 (29.6%) of those deaths were ascribed to CV-related causes. No significant associations were observed among E2, all-cause mortality (HR = 1.05; 95% CI = 0.89-1.23 per 1 – SD E2 increase), and CV mortality (HR = 0.99; 95% CI = 0.69-1.41 per 1 – SD E2 increase) after adjustment. Among 234 participants without prior CV events, 80 (34.2%) had an incident CVD event. No significant linear associations were observed between E2 and incident CVD, even after considering competing events (HR = 1.01; 95% CI = 0.83-1.22 per 1 – SD E2 increase). Age and diabetes did not significantly modify these associations (interaction *P* all ≥ .208).

We saw that models with a quadratic term for E2 might produce a better fit compared with the linear models. The Bayesian information criterion values for the quadratic models were consistently lower than those from the linear models for all-cause mortality, CVD mortality, and incident CVD. E2 showed a concave association with all-cause mortality (nonlinear $P = .022$): E2 levels below 200 pmol/L are positively associated with all-cause mortality risk, while no association or potentially a very weak inverse association was suggested for E2 levels above 200 pmol/L (Figure 2A). Although quadratic models were a better fit for our data, no significant nonlinear associations were observed between E2 and CVD mortality (nonlinear $P = .163$), or incident CVD (nonlinear $P = .385$) (Figure 2B and C).

E2 was not significantly associated with HUI3 scores (MD = 0.008; 95% CI = –0.015 to 0.031 per 1 – SD E2 increase) (Table 2). However, age modified this association ($P = .045$). Among patients aged ≥63 years, higher E2 levels were associated with lower HUI3 scores, MD = –0.062 per 1 – SD E2 increase, 95% CI = –0.112 to –0.012, but the opposite was observed in younger patients (<63 years) in whom higher E2 levels were associated with higher HUI3 scores (MD = 0.032 per 1 – SD E2 increase, 95% CI = 0.008-0.055) (Table 3).

No significant associations were observed between E2 and KDQOL12-PCS (MD = –0.15; 95% CI = –1.15 to 0.86 per 1 – SD E2 increase) or KDQOL12-MCS scores (MD = –0.63; 95% CI = –1.82 to 0.57 per 1 – SD E2 increase) (Table 2). No other significant effect modification by age or diabetes was found (all interactions $P \geq .176$).

During sensitivity analysis, women in the highest E2 tertile had higher all-cause mortality risk (HR =1.61, 95% CI =

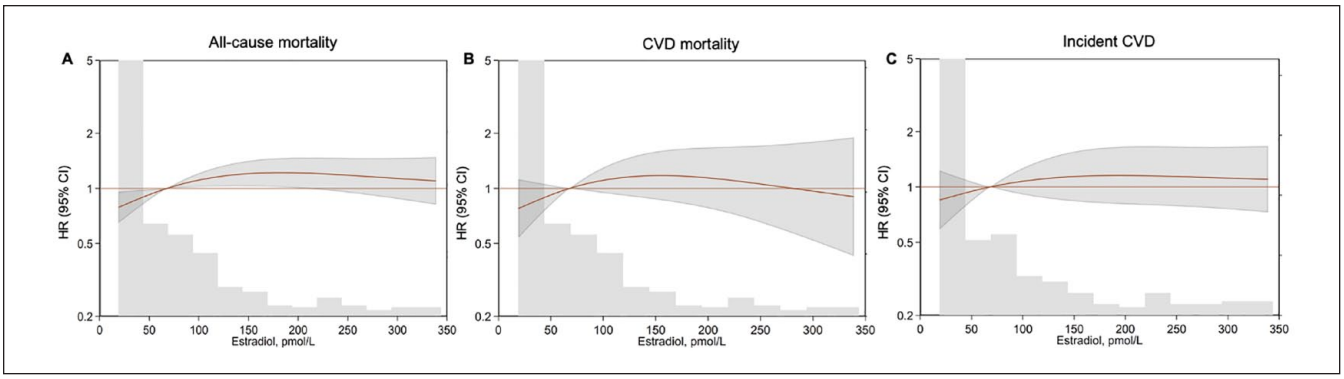

Figure 2. Nonlinear associations between E2, all-cause mortality, CVD mortality, and incident CVD.
Note. Nonlinear associations between (A) E2 and all-cause mortality (nonlinearity $P = .022$), (B) E2 and CVD mortality (nonlinearity $P = .163$), and (C) E2 and incident CVD (nonlinearity $P = .385$). In (C), the plateau in spline function at low E2 levels is likely due to the exclusion of older participants with a prior CV event. When older participants with a prior CV event were included, the spline function in Figure 2C bore closer resemblance to those of (A) and (B) (not shown). Nonlinearity was investigated by introducing a linear and quadratic term to the models with all-cause and CV mortality, as well as incident CVD outcomes. Solid line represents the estimated HRs of the spline function for all-cause mortality, CV mortality, and incident CVD. Shaded gray area represents the 95% CI of the spline HR estimation, respectively. The histograms illustrate the population density of the E2 concentrations. E2 = estradiol; CVD = cardiovascular disease; CV = cardiovascular; HR = hazard ratio; CI = confidence interval.

Table 2. Adjusted Mean Differences for HRQOL Measures per 1 – SD Higher Level of E2.

Instrument	No of measures (participants)	MD (95% CI)
HUI3	1251 (378)	0.008 (−0.015 to 0.031)[a]
KDQOL12–PCS	716 (300)	−0.145 (−1.149 to 0.859)
KDQOL12–MCS	716 (300)	−0.627 (−1.821 to 0.567)

Note. MD (95% CI) calculated per 1 – SD increment of E2 and adjusted for age, BMI, systolic blood pressure, ethnicity (white/Indigenous/other), glomerulonephritis, diabetes, CVD (stroke, transient ischemic attack, coronary artery disease, heart failure, and peripheral vascular disease), mental health (smoking status, substance misuse, and psychiatric disorder), other serious illnesses (cancer, chronic respiratory disease, chronic liver disease, and dementia), and albumin. Participants were modeled as random effects and visit timepoints (ie, baseline, month 6, years 1, 2, 5, and 10) as fixed effects. HRQOL = health-related quality of life; E2 = estradiol; MD = mean difference; CI = confidence interval; HUI3 = Health Utilities Index Mark 3 scores; KDQOL12-PCS = Kidney Disease Quality of Life Physical Component Score; KDQOL12-MCS = Kidney Disease Quality of Life Mental Component Score; BMI = body mass index; CVD = cardiovascular disease.
[a]*P* value for interaction with age = .045. Age was dichotomized into < 63 and ≥63 years.

Table 3. Adjusted Mean Differences for HUI3 Scores by Age per 1 – SD Higher Level of E2.

Age	No of measures (participants)	MD (95% CI)
<63 years	601 (187)	0.032 (0.008 to 0.055)
≥63 years	650 (191)	–0.062 (–0.112 to –0.012)

Note. MDs (95% CI) calculated per 1 – SD E2 increase and adjusted for age, BMI, systolic blood pressure, ethnicity (white/Indigenous/other), glomerulonephritis, diabetes, CVD (stroke, transient ischemic attack, coronary artery disease, heart failure, and peripheral vascular disease), mental health (smoking status, substance misuse, and psychiatric disorder), other serious illnesses (cancer, chronic respiratory disease, chronic liver disease, and dementia) and albumin. HUI3 = Health Utilities Index Mark 3 scores; E2 = estradiol; MD = mean difference; CI = confidence interval; BMI = body mass index; CVD = cardiovascular disease.

1.16-2.23) compared with women in the lowest E2 tertile. No significant associations were observed between E2 tertiles, CVD mortality, and incident CVD.

Discussion

The current study attempted to delineate associations among E2, mortality, and HRQOL using data from a prospective cohort of 427 women receiving maintenance HD in Canada. Over a median follow-up of 3.6 years (IQR = 1.6-7.5 years), significant concave associations were observed between E2 levels and all-cause mortality, but not with CVD mortality and incident CVD. Furthermore, E2 levels were not associated with HUI3, KDQOL12-PCS, or KDQOL12-MCS scores. However, the association between E2 and HUI3 scores was significantly modified by age. For every 1 – SD increase in serum E2, HUI3 scores were higher for younger women (<63 years), but were lower among older women (≥63 years). No other associations reported herein were modified by age or diabetes.

Ramesh et al[13] used the same database to analyze the relation between E2 and mortality, and found that over a shorter mean follow-up period of 2.9 years, women in the 2 highest E2 quintiles had higher all-cause mortality risk compared with those in the lowest quintile. Another study by Tanrisev et al[9] reported a U-shaped association among E2, all-cause mortality, and CVD mortality over a 3-year follow-up period among 283 women receiving maintenance HD. Both studies reported associations between higher E2 levels and increased

all-cause mortality risk,[9,13] consistent to our observations. However, the concave association between E2 and all-cause mortality contradicts with abovementioned observations of Tanrisev et al.[9] This discrepancy could be potentially attributed to the inclusion of younger participants in our study (≥18 years old), compared with Tanrisev et al[9] where only women >45 years old were included. Furthermore, Tanrisev et al measured serum E2 levels in participants who were on dialysis for >6 months, whereas E2 levels in our study were measured within 3 months of starting HD.

The mechanism for the putative association between E2 and mortality (in the general population and/or HD patients) is not well understood, although could be related to effects on the CV system or inflammation.[31] E2 deficiency could promote vascular calcification[32,33] and inflammation.[34-36] While proinflammatory cytokines can inhibit gonadal E2 production, it can stimulate aromatase activity to induce peripheral conversion of androgens to estrogens.[37] In critically ill patients with high inflammatory burden,[9,38] higher serum E2 levels have been linked with increased mortality[9,13,37,39-41]—potentially explaining a portion of the nonlinear association characterized by a positive association between E2 and all-cause mortality. The other portion toward the right-hand side, characterized by wide CIs suggesting either no association or potentially a very weak inverse association between E2 and all-cause mortality (Figure 2A), could be due to the low proportion of women with higher E2 levels. Nevertheless, causality or pathophysiological significance cannot be demonstrated due to the observational nature of these studies (including ours). Higher E2 levels among women who died do not necessarily indicate a harmful excess. Conversely, elevated E2 may not have pathophysiological significance. Higher E2 levels may simply reflect a state of poor health, and its associations with mortality in the kidney population remains unclear. Nevertheless, the null associations between E2 levels with CVD mortality and incident CVD in the current study might be due to the relatively short follow-up time (median = 3.6 years) and low number of participants reaching these endpoints.

In a 12-month study involving women aged between 18 and 45 years with kidney failure on HD with E2 levels <30 pg/mL (<110 pmol/L) and secondary amenorrhea, women given transdermal 17β-estradiol and cyclic addition of norethisterone had significantly increased mean E2 levels (from 20.5 to 46.8 pg/mL or 75 to 172 pmol/L), resumed regular menstruation, showed lower prolactin levels, and reported significantly better libido and HRQOL compared with controls.[20] These findings are consistent with our observations that for younger women on HD, higher HUI3 scores were significantly associated with higher E2 levels. In contrast, among older women on HD, lower HUI3 scores were significantly associated with higher E2 levels. This may be due to the higher prevalence of comorbidities such as CVD and diabetes among the older population, known to negatively impact HRQOL in HD patients.[6] Building on this, older women on HD in our study may have experienced a longer period of reduced EEE prior to HD initiation compared with younger women. Endogenous estrogen exposure and early menopause have been associated with increased CVD mortality,[11] lower physical health, and lower psychological well-being,[12] leading to lower HRQOL. Although the association between E2 levels and HUI3 scores among younger and older women suggest clinically important changes,[27] contextualizing minimal clinically important scores remains challenging in patients with kidney disease. Therefore, more research is needed in this area.

In a placebo-controlled randomized trial in 3721 postmenopausal women, treatment with 0.625 mg conjugated equine estrogen plus 2.5/5.0 mg medroxyprogesterone conferred small but significant improvements in HRQOL.[42] Another trial in 2763 postmenopausal women given the same treatment reported mixed effects on HRQOL, depending on whether menopausal symptoms were present: Women with flushing had improved emotional measures, whereas women without flushing had worse physical measures.[43] However, these studies were conducted among women without kidney disease. Reports on HRT usage among women with kidney failure only included surrogate outcomes.[44] Therefore, studies are still needed to assess the effects of HRT on women with kidney disease as data are still lacking.

Diabetes has been associated with altered sex hormone levels, worse cardiometabolic profile, and increased mortality risk.[7,45] Reports conflict concerning E2 levels in women with diabetes in comparison with those without diabetes,[45-47] and may not extend to those with kidney disease. Furthermore, in advanced stages of diabetes with kidney failure, the relationships of serum E2 and study outcomes might be blurred by morbidity burden and severity of kidney failure. This could explain why we did not observe effect modification by diabetes status on the relationships between serum E2 concentrations and study outcomes.

As amenorrhea is common among women on HD, ascertaining menopausal status among this patient population remains challenging.[23,48] Although menopause is defined as the secondary absence of menses for at least 12 months,[49] amenorrhea among women with kidney disease does not necessitate a postmenopausal classification as amenorrhea can be reversed with continued HD or kidney transplantation.[50,51] Due to menstrual cycle variation, E2 levels of healthy premenopausal women can range between 73.4 and 2753.5 pmol/L.[52] However, E2 levels of premenopausal women on dialysis do not vary as expected,[3,23] and can also be seen in the current study as the 75th percentile of the highest E2 tertile is 294 pmol/L (Table 3). As such, our findings are solely based on E2 levels and age.

Our study has several strengths, including data from a well-characterized HD population and a prospective, multicenter design. We were able to ascertain which participants underwent gynecological procedures and adjust for multiple confounders such as malignancies and chronic respiratory

disorders. Also, our data were collected over a relatively long follow-up period (≈14 years). Furthermore, we were able to include data for HRQOL.

Our study also has some limitations. E2 is primarily bound to sex hormone–binding globulin (SHBG), and only free fractions can bind receptors and elicit physiological responses. The unavailability of SHBG measurements precluded estimation of free E2 levels in our study. Furthermore, hormonal diagnostic cut-offs typically used to determine menopausal status are not reliable for women with kidney failure,[13] as the hormonal-hypothalamic pituitary axis is disturbed by progressive kidney disease and eventual failure.[53] Thus, the impact of menopausal status on the associations between E2 and assessed outcomes could not be evaluated in the present study. Another limitation is that we did not collect information on sex or intersex categorizations in the current study. In addition, patients dropped out over time, so we had insufficient power to address effect modification by diabetes status and outcomes of interest. Moreover, because a single E2 measurement at baseline was used, we could not monitor changes in E2 levels over time and evaluate its effects with our outcomes of interest. Nevertheless, it has been previously shown that single sex hormone measurements can adequately represent long-term sex hormone levels.[54] We also excluded participants with missing data. As those excluded were not significantly different from included participants (Supplemental Table S1), the exclusions are unlikely to have affected our study findings. Finally, despite our efforts to adjust for known risk factors, we were unable to adjust for C-reactive protein (an inflammation marker)[55] and frailty.[56] In addition, residual confounding is possible due to the nature of observational studies.

Conclusions

Data from a well-characterized prospective cohort of 427 women on HD in Canada suggest that the association between E2 and all-cause mortality may be nonlinear. Furthermore, E2 was not significantly associated with CVD mortality, incident CVD, KDQOL12-PCS, and KDQOL12-MCS. However, data for the relationship between E2 levels and HUI3 were mixed, with higher E2 levels associated with higher HUI3 scores among younger women (<63 years), and with lower HUI3 scores among older women (≥63 years). Diabetes status did not modify the relationships. Therefore, further work is warranted on the associations of E2 and adverse health outcomes among women with kidney failure on HD.

Ethics Approval and Consent to Participate

Written informed consent was obtained and relevant research ethics boards approved the study (Pro00002385, REB15-1048).

Consent for Publication

All listed authors herein consented to the publication of this paper.

Availability of Data and Materials

The data are not publicly available due to national data protection laws and restrictions imposed by relevant research ethics boards to ensure data privacy of the study participants. However, the data that support the findings of this study are available upon reasonable request from the Research Manager for the Kidney Health Research Group, Natasha Wiebe.

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Author Contributions

L.L. and A.K.B. developed the research idea and study design. L.L. and N.W. developed the statistical analysis plan. L.L. wrote the manuscript with support from N.W. and A.K.B. N.W., S.R., S.A., S.K., J.-J.C., P.Stenvinkel, M.T., and A.K.B. acquired the data. N.W. analyzed the data and its interpretation was supported by L.L., N.W., S.R., S.A., S.K., J.-J.C., P.Stenvinkel, P.Senior, M.T., A.B., and B.T. A.K.B. is the guarantor of this work and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors provided critical feedback, helped shape the manuscript, and approved the final version of the manuscript.

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Supplemental Material

Supplemental material for this article is available online.

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