BMJ Open Diabetes Research & Care

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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To cite: Lau LHY, Nano J, Cecil A, *et al*. Cross-sectional and prospective relationships of endogenous progestogens and estrogens with glucose metabolism in men and women: a KORA F4/FF4 Study. *BMJ Open Diab Res Care* 2021;**9**:e001951. doi:10.1136/bmjdrc-2020-001951

► Supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2020-001951).

Received 15 October 2020
Revised 3 January 2021
Accepted 9 January 2021

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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

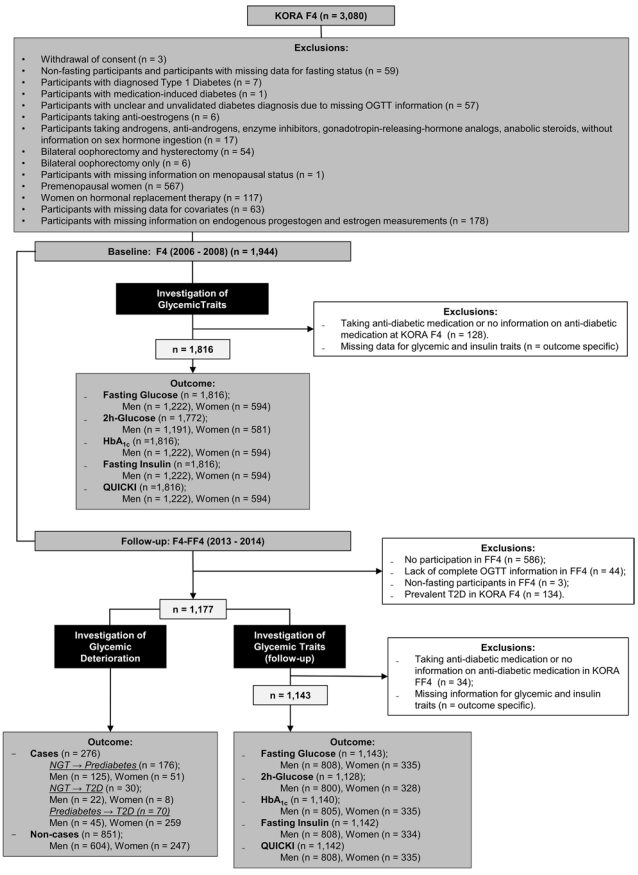

Figure 1 Flowchart showing sample sizes and exclusions. HbA_{1c}, glycated hemoglobin; KORA, Cooperative Health Research in the Region of Augsburg; NGT, normoglycemia; OGTT, oral glucose tolerance test; QUICKI, Quantitative Insulin Sensitivity Check Index; T2D, type 2 diabetes.

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).

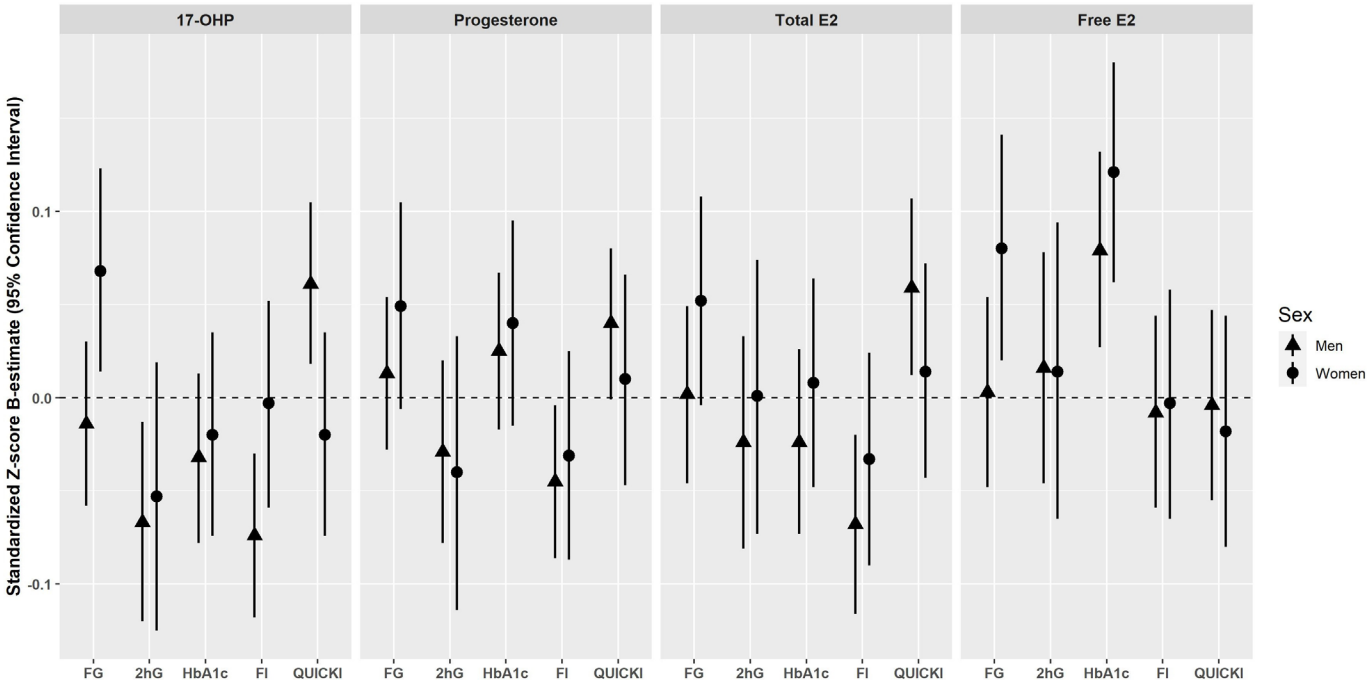

Figure 2 Cross-sectional associations of endogenous progestogens and estrogens with glycemic traits in men and women of the KORA F4 cohort*. Results are expressed as the change in 1 log unit of the continuous outcome (standardized Z-score β-estimate with 95% CI) per 1 sex-specific SD increase in the 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

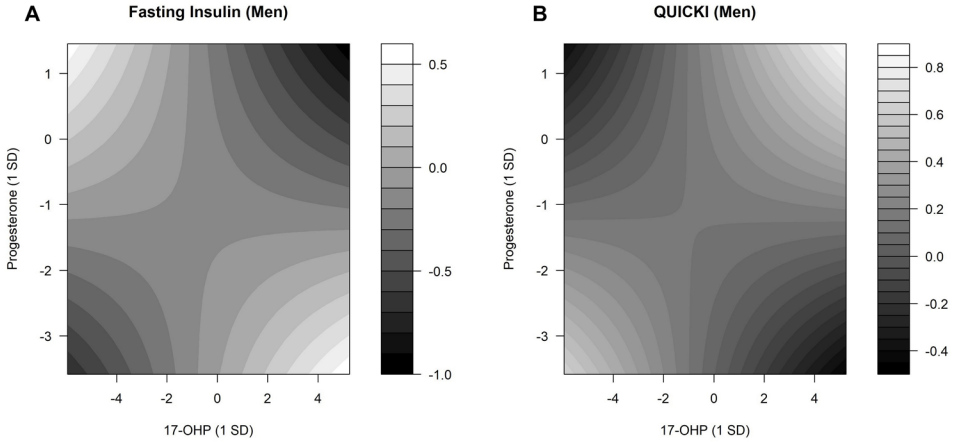

Figure 3 Interaction effects between 17-OHP and progesterone regarding fasting serum insulin and QUICKI. Contour plots estimated by linear regression models demonstrate the changes in fasting insulin and QUICKI for different concentrations of 17-OHP and progesterone. The predicted fasting serum insulin 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.

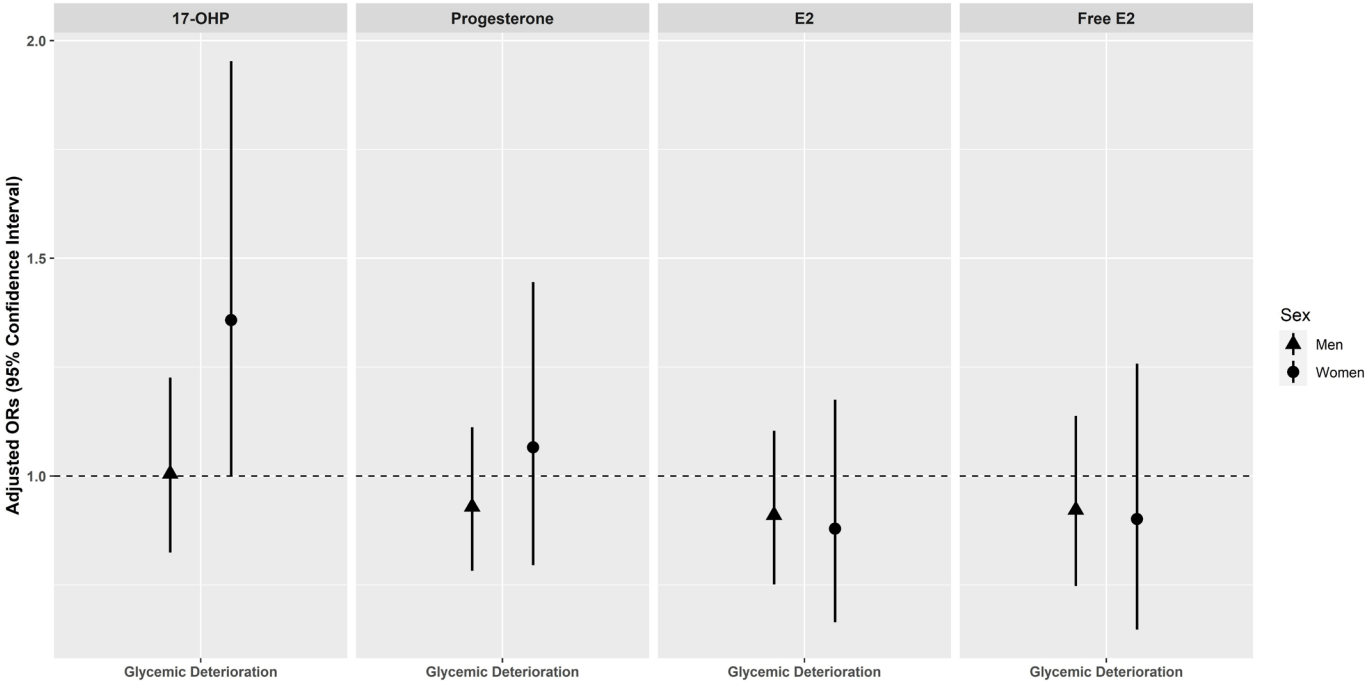

Figure 4 Association of endogenous progestogens and estrogens with glycemic deterioration in men and women of the KORA F4/FF4 cohort*. Adjusted ORs with 95% CIs for glycemic deterioration per 1 sex-specific SD increase in log-transformed progestogen and estrogen. ORs are adjusted for baseline age, waist 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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Acknowledgements We thank the members of the Research Unit Molecular Endocrinology and Metabolism, Helmholtz Zentrum München, Germany, for their excellent technical work in sample preparation and quantification. We also extend our gratitude to all members of the Institute of Epidemiology, Helmholtz Zentrum München, and the KORA field staff in Augsburg who planned and conducted the study.

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.

Funding This study was supported in part by a research grant within the German Center for Cardiovascular Research (DZHK) shared expertise program (DZHK B 17-035 SE) and by funding from the Helmholtz Alliance 'Aging and Metablic Programming, AMPro'. LHYL is supported through the International Helmholtz Research School for Diabetes. TZ is supported by the German Center for Cardiovascular Research (DZHK81Z1710101, FKZ81Z0710102, and FKZ 81X2710160). The Cooperative Health Research in the Region of Augsburg study was initiated and financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria.

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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Supplementary Figure 1 – Ascertainment of the glycemic deterioration outcome in men and women of KORA F4/FF4.

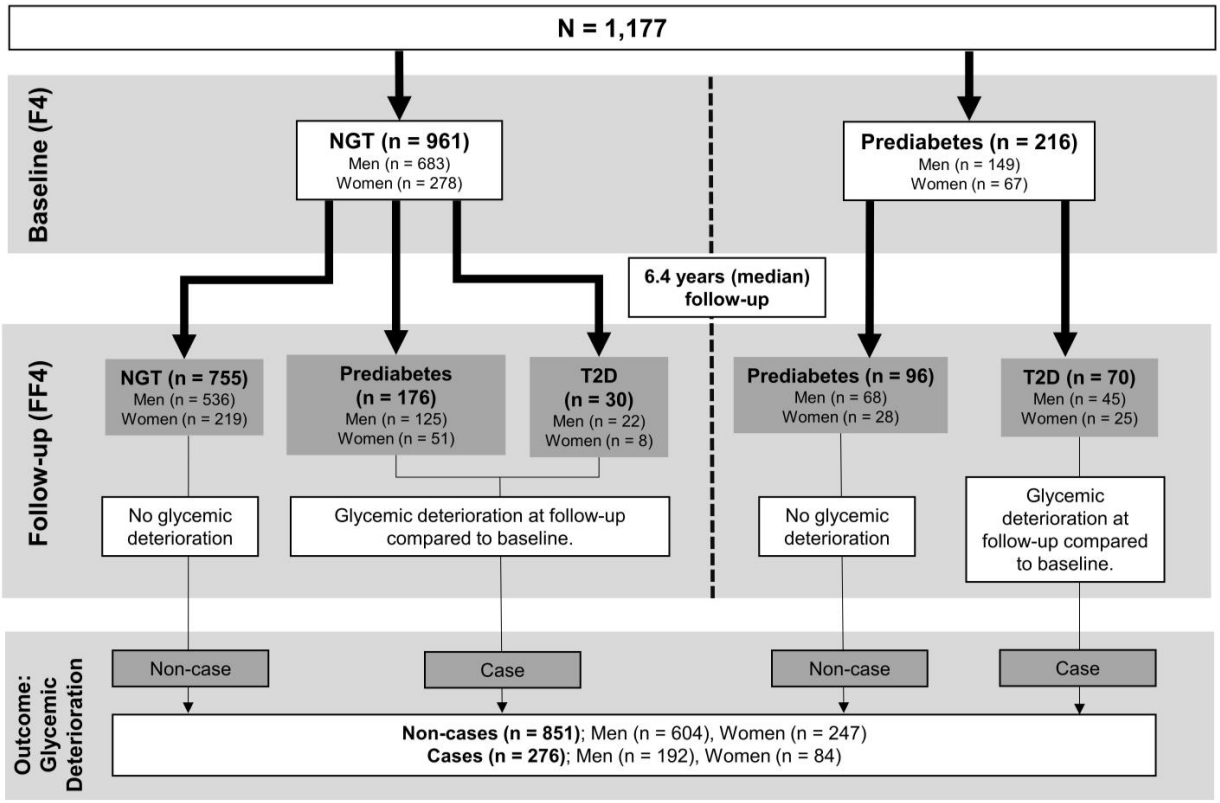
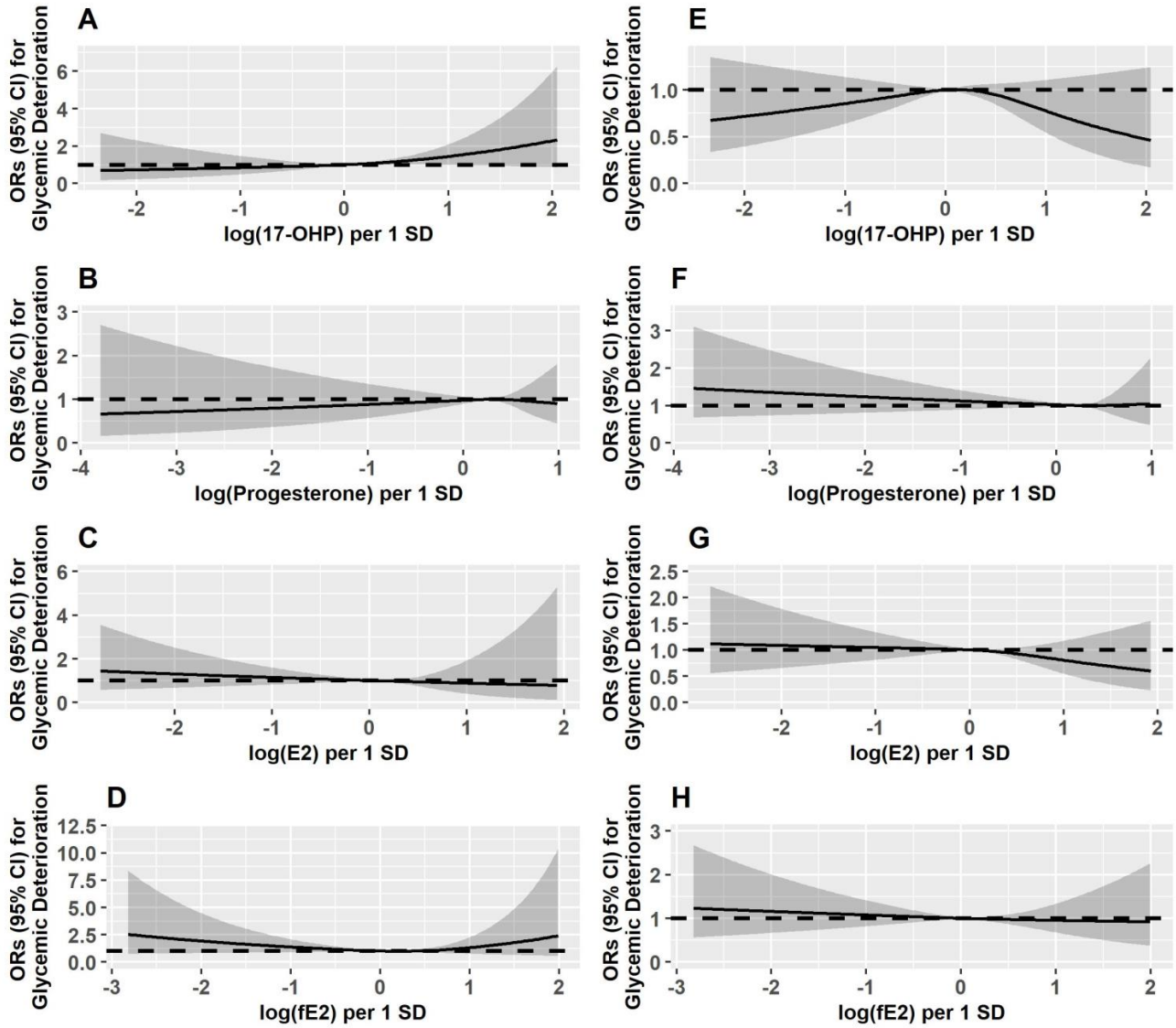


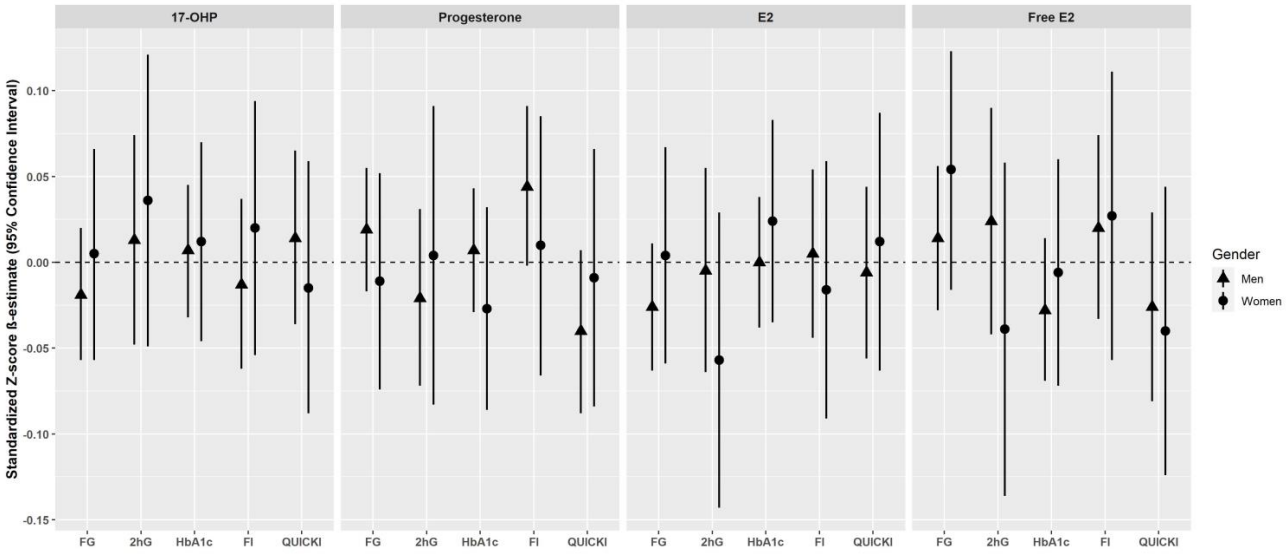
Illustration of the classification of participants with glycemic deterioration over the follow-up period. Non-cases were those who had normal glucose tolerance (NGT) at baseline (F4) and at follow-up (FF4) or who had prediabetes at baseline (F4) and follow-up (FF4). Cases were those who progressed from NGT at baseline to either prediabetes or T2D at follow-up or who progressed from prediabetes at baseline to T2D at follow-up.

Supplementary Figure 2 – Non-linear associations of endogenous progestogens and estrogens with glycemic deterioration in men and women of KORA F4/FF4.



Non-linear relationships between sex hormones and glycemic deterioration in men (A, B, C, and D) and women (E, F, G, and H). Multivariate adjusted logistic regression models exploring sex hormone levels and associations with glycemic deterioration were entered into the model as a restricted cubic spline. Knots were placed at the 30th, 60th, and 90th percentiles. The shaded grey area represents the 95% CI of the spline estimation. *Adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol/HDL-cholesterol ratio, hypertension, statin use, smoking, alcohol consumption, physical activity, CRP, eGFR (creatinine-based), TSH, and parental diabetes history. Additionally, models for progesterone are adjusted for albumin, and models for E2 are adjusted for SHBG. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, SHBG: Sex hormone-binding globulin, TSH: Thyroid-stimulating hormone. P-values for spline fit are described in Supplementary Table 5.*

Supplementary Figure 3 – Prospective associations of endogenous progestogens and estrogens with T2D-related traits in men and women of the KORA F4/FF4 cohort*.



*Results are expressed as the change in one log-unit of the continuous outcome (standardized Z-score β-estimate with 95% CI) per one sex-specific SD increase in the respective progestogens and estrogens. Adjusted for baseline values of respective glycemic traits, waist circumference, height, triglycerides, total cholesterol/HDL-cholesterol ratio, hypertension, statin use, smoking status, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental history of diabetes (model 2). FG: Fasting glucose; 2hG: 2h-glucose; HbA_{1c}: Glycated hemoglobin; FI: Fasting insulin; QUICKI: Quantitative Insulin Sensitivity Check Index, eGFR: estimated glomerular filtration rate, TSH: Thyroid-stimulating hormone. *Men and peri-/postmenopausal women who did not take anti-diabetic medication.*

Supplementary Table 1 – P-values for sex interactions in associations between endogenous progestogens and estrogens with glycemic traits and glycemic deterioration in the KORA F4/FF4 study.

	P-value for sex interaction			
	17-OHP	**Progesterone**	**E2**	**Free E2**
Fasting glucose	0.095	0.445	0.306	0.139
2h-glucose	0.829	0.860	0.799	0.506
HbA_{1c}	0.545	0.929	0.714	0.051
Fasting insulin	**0.004**	0.149	**0.027**	**0.008**
QUICKI	**0.005**	0.104	**0.036**	**0.007**
Glycemic deterioration	0.152	0.412	0.621	0.547

P-values for interaction between sex and sex hormones on continuous glycemic outcomes in cross-sectional linear regression models and glycemic deterioration in prospective logistic regression models. Adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol/HDL-cholesterol ratio), hypertension, statin use (model 1), smoking, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental diabetes history (model 2). Sex interaction was entered as a multiplicative term in model 2.

Supplementary Table 2 - Cross-sectional associations of endogenous progestogens and estrogens with glycemic traits in men of KORA F4.

		17-OHP	Progesterone	Progesterone[a]	E2	E2[b]	fE2
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Fasting Glucose	**Model 1**	-0.019 (-0.064 – 0.026) P = 0.410	0.017 (-0.025 – 0.059) P = 0.438	-	-0.014 (-0.063 – 0.035) P = 0.570	-	0.038 (-0.014 – 0.090) P = 0.153
	Model 2	-0.014 (-0.058 – 0.030) P = 0.529	0.013 (-0.028 – 0.054) P = 0.544	0.011 (-0.030 – 0.051) P = 0.606	0.002 (-0.046 – 0.049) P = 0.947	0.003 (-0.047 – 0.052) P = 0.913	0.003 (-0.048 – 0.054) P = 0.920
2h-glucose	**Model 1**	**-0.074 (-0.130 – -0.019) P = 0.009**	-0.024 (-0.075 – 0.027) P= 0.354	-	**-0.059 (-0.118 – -0.001) P = 0.048**	-	0.039 (-0.024 – 0.103) P = 0.225
	Model 2	**-0.067 (-0.120 – -0.013) P = 0.014**	-0.029 (-0.078 – 0.020) P = 0.242	-0.030 (-0.079 – 0.019) P = 0.228	-0.024 (-0.081 – 0.033) P = 0.413	-0.013 (-0.073 – 0.046) P = 0.660	0.016 (-0.046 – 0.078) P = 0.609
HbA_{1c}	**Model 1**	-0.041 (-0.087 – 0.006) P = 0.088	0.021 (-0.022 – 0.065) P = 0.340	-	-0.048 (-0.098 – 0.002) P = 0.061	-	**0.086 (0.032 – 0.140) P = 0.002**
	Model 2	-0.032 (-0.078 – 0.013) P = 0.163	0.025 (-0.017 – 0.067) P = 0.248	0.028 (-0.015 – 0.070) P = 0.199	-0.024 (-0.073 – 0.026) P = 0.350	0.012 (-0.039 – 0.063) P = 0.648	**0.079 (0.027 – 0.132) P = 0.003**
Fasting Insulin	**Model 1**	**-0.093 (-0.140 – -0.046) P < 0.001**	**-0.052 (-0.096 – -0.008) P = 0.020**	-	**-0.113 (-0.163 – -0.062) P < 0.001**	-	0.013 (-0.041 – 0.068) P = 0.628
	Model 2	**-0.074 (-0.118 – -0.030) P = 0.001**	**-0.045 (-0.086 – -0.004) P = 0.031**	**-0.047 (-0.088 – -0.006) P = 0.026**	**-0.068 (-0.116 – -0.020) P = 0.006**	**-0.055 (-0.105 – -0.005) P = 0.030**	-0.008 (-0.059 – 0.044) P= 0.771
QUICKI	**Model 1**	**0.079 (0.032 – 0.126) P = 0.001**	**0.045 (0.001 – 0.088) P = 0.046**	-	**0.105 (0.054 – 0.155) P < 0.001**	-	-0.031 (-0.085 – 0.024) P = 0.271
	Model 2	**0.061 (0.018 – 0.105) P = 0.006**	0.040 (-0.001 – 0.080) P = 0.057	**0.041 (0.001 – 0.082) P = 0.046**	**0.059 (0.012 – 0.107) P = 0.015**	0.044 (-0.005 – 0.093) P = 0.079	-0.004 (-0.055 – 0.047) P = 0.888

All results are from multivariate linear regression models. Adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol/HDL-cholesterol ratio), hypertension, statin use (model 1), smoking, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental diabetes history (model 2). Effect estimates with 95% CIs were calculated for a one sex-specific SD increase on the log scale of progestogen and estrogen levels, respectively. Significant results are printed in bold. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, SHBG: Sex hormone-binding globulin, TSH: Thyroid-stimulating hormone.

[a] *Models were additionally adjusted for albumin*

[b] *Models were additionally adjusted for SHBG.*

Supplementary Table 3 - Cross-sectional associations of endogenous progestogens and estrogens with glycemic traits in women of KORA F4.

		17-OHP	Progesterone	Progesterone[a]	E2	E2[b]	fE2
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Fasting Glucose	Model 1	**0.071 (0.015 – 0.127) P = 0.013**	0.042 (-0.015 – 0.099) P = 0.148	-	0.049 (-0.008 – 0.106) P = 0.093	-	**0.094 (0.033 – 0.156) P = 0.003**
	Model 2	**0.068 (0.014 – 0.123) P = 0.014**	0.049 (-0.006 – 0.105) P = 0.081	0.054 (-0.001 – 0.109) P = 0.054	0.052 (-0.004 – 0.108) P = 0.067	0.055 (-0.000 – 0.111) P = 0.051	**0.080 (0.020 – 0.141) P = 0.009**
	Model 2 (Sens.)[c]	**0.098 (0.032 – 0.164) P = 0.004**	**0.071 (0.007 – 0.136) P = 0.031**	-	**0.076 (0.014 – 0.137) P = 0.016**	-	**0.092 (0.026 – 0.159) P = 0.007**
2h-glucose	Model 1	-0.056 (-0.130 – 0.017) P = 0.131	-0.050 (-0.124 – 0.025) P = 0.190	-	-0.001 (-0.076 – 0.074) P = 0.974	-	0.028 (-0.053 – 0.108) P = 0.502
	Model 2	-0.053 (-0.125 – 0.019) P = 0.148	-0.040 (-0.114 – 0.033) P = 0.278	-0.036 (-0.109 – 0.037) P = 0.334	0.001 (-0.073 – 0.074) P = 0.987	0.001 (-0.072 – 0.075) P = 0.975	0.014 (-0.065 – 0.094) P = 0.725
	Model 2 (Sens.)[c]	-0.057 (-0.145 – 0.031) P= 0.201	-0.030 (-0.117 – 0.056) P = 0.492	-	0.017 (-0.065 – 0.098) P = 0.690	-	0.018 (-0.070 – 0.107) P = 0.682
HbA_{1c}	Model 1	-0.022 (-0.076 – 0.033) P = 0.438	0.032 (-0.023 – 0.087) P = 0.252	-	0.004 (-0.052 – 0.060) P = 0.888	-	**0.123 (0.064 – 0.182) P < 0.001**
	Model 2	-0.020 (-0.074 – 0.035) P = 0.481	0.040 (-0.015 – 0.095) P = 0.154	0.040 (-0.015 – 0.095) P = 0.150	0.008 (-0.048 – 0.064) P = 0.777	0.019 (-0.034 – 0.072) P = 0.481	**0.121 (0.062 – 0.180) P < 0.001**
	Model 2 (Sens.)[c]	0.007 (-0.057 – 0.071) P = 0.832	**0.071 (0.008 – 0.133) P = 0.026**	-	0.033 (-0.026 – 0.093) P = 0.271	-	**0.139 (0.076 – 0.202) P < 0.001**
Fasting Insulin	Model 1	-0.002 (-0.059 – 0.056) P = 0.959	-0.033 (-0.092 – 0.026) P = 0.268	-	-0.035 (-0.094 – 0.024) P = 0.244	-	0.008 (-0.056 – 0.071) P = 0.810
	Model 2	-0.003 (-0.059 – 0.052) P = 0.907	-0.031 (-0.087 – 0.025) P = 0.279	-0.027 (-0.083 – 0.029) P = 0.343	-0.033 (-0.090 – 0.024) P = 0.260	-0.030 (-0.088 – 0.027) P = 0.298	-0.003 (-0.065 – 0.058) P = 0.913
	Model 2 (Sens.)[c]	-0.005 (-0.072 – 0.062) P = 0.884	-0.032 (-0.097 – 0.033) P = 0.336	-	-0.013 (-0.075 – 0.050) P = 0.692	-	0.008 (-0.059 – 0.075) P = 0.814
QUICKI	Model 1	-0.021 (-0.079 – 0.036) P = 0.466	0.015 (-0.045 – 0.075) P = 0.625	-	0.018 (-0.043 – 0.078) P = 0.568	-	-0.033 (-0.098 – 0.032) P = 0.316
	Model 2	-0.020 (-0.074 – 0.035) P = 0.486	0.010 (-0.047 – 0.066) P = 0.742	0.004 (-0.052 – 0.061) P = 0.879	0.014 (-0.043 – 0.072) P = 0.622	0.011 (-0.047 – 0.068) P = 0.713	-0.018 (-0.080 – 0.044) P = 0.562
	Model 2 (Sens.)[c]	-0.027 (-0.094 – 0.040) P = 0.433	0.005 (-0.060 – 0.071) P = 0.877	-	-0.012 (-0.074 – 0.051) P = 0.717	-	-0.034 (-0.101 – 0.034) P = 0.327

All results are from multivariate linear regression models. Adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol/HDL-cholesterol ratio, hypertension, statin use (model 1), smoking, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental diabetes history (model 2). Effect estimates with 95% CIs were calculated for a one sex-specific SD increase on the log scale of progestogen and estrogen levels, respectively. Significant results are printed in bold. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, SHBG: Sex hormone-binding globulin, TSH: Thyroid-stimulating hormone.

[a] *Models were additionally adjusted for albumin*
[b] *Models were additionally adjusted for SHBG.*
[c] *Sensitivity analyses: Perimenopausal women excluded (n = outcome specific).*

Supplementary Table 4 – Prospective associations of endogenous progestogens and estrogens with glycemic deterioration.

		Men		Women	
		Glycemic Deterioration *Cases (n = 193), non-cases (n = 604)*		**Glycemic Deterioration** *Cases (n = 85), non-cases (n = 247)*	
		OR (95% CI)	**P value**	**OR (95% CI)**	**P-value**
17-OHP	Model 1	0.969 (0.802 – 1.172)	0.746	1.255 (0.950 – 1.661)	0.110
	Model 2	1.005 (0.824 – 1.226)	0.961	1.358 (0.999, 1.853)	0.051
	Model 2 (Sens.)*	-	-	**1.518 (1.033, 2.264)**	**0.036**
Progesterone	Model 1	0.934 (0.795 – 1.105)	0.413	0.983 (0.756 – 1.296)	0.902
	Model 2	0.929 (0.782 – 1.112)	0.412	1.066 (0.795 – 1.445)	0.675
	Model 2 + Albumin**	0.926 (0.779 – 1.108)	0.388	1.043 (0.778 – 1.414)	0.780
	Model 2 (Sens.)*	-	-	1.168 (0.813 – 1.725)	0.414
E2	Model 1	0.899 (0.750 – 1.078)	0.246	0.865 (0.671 – 1.128)	0.267
	Model 2	0.910 (0.751 – 1.104)	0.337	0.879 (0.664 – 1.175)	0.371
	Model 2 + SHBG**	0.909 (0.743 – 1.112)	0.352	0.890 (0.668 – 1.199)	0.431
	Model 2 (Sens.)*	-	-	0.893 (0.662 – 1.219)	0.465
fE2	Model 1	0.970 (0.797 – 1.183)	0.760	0.907 (0.664 – 1.245)	0.542
	Model 2	0.922 (0.747 – 1.138)	0.448	0.901 (0.647 – 1.258)	0.535
	Model 2 (Sens.)*	-	-	0.851 (0.592 – 1.223)	0.380

*All results are from a multivariate logistic regression. Model 1: Adjusted for baseline age, waist circumference, and height. Model 2: Model 1 + triglycerides, total cholesterol/HDL-cholesterol ratio, hypertension, statin use, smoking, alcohol consumption, physical activity, CRP, eGFR (creatinine-based), TSH, and parental diabetes history. ORs with 95% CIs for worse glycemic outcomes were calculated for a one sex-specific SD increase on the log scale of progestogen and estrogen levels, respectively.*Sensitivity analysis where perimenopausal women (n = 66; 15 cases and 51 non-cases) were excluded. **Further adjustment of progesterone with albumin and E2 with SHBG. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, TSH: Thyroid-stimulating hormone.*

Supplementary Table 5 – Non-linear associations of endogenous progestogens and estrogens with glycemic deterioration.

Sex Hormones	P-value for non-linear fit		
	Men	**Women**	**Women****
17-OHP	0.103	0.568	0.356
Progesterone	0.794	0.665	0.344
Total E2	0.464	0.997	0.696
Free E2	0.922	0.108	0.324

Multivariate adjusted logistic regression models exploring sex hormone levels and associations with glycemic deterioration were entered into the model as a restricted cubic spline. Knots were placed at the 30th, 60th, and 90th percentiles. The shaded grey area represents the 95% CI of the spline estimation. *Adjusted for age, waist circumference, height, triglycerides, total cholesterol/HDL-cholesterol ratio, hypertension, statin use, smoking, alcohol consumption, physical activity, CRP, eGFR (creatinine-based), TSH, and parental diabetes history. Additionally, models for progesterone are adjusted for albumin, and models for E2 are adjusted for SHBG. **Sensitivity analyses performed where perimenopausal women (n = 66; 15 cases and 51 non-cases) were excluded. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, SHBG: Sex hormone-binding globulin, TSH: Thyroid-stimulating hormone.*

Supplementary Table 6 – P-values for interaction terms between 17-OHP and progesterone in multivariate linear regression models examining associations between sex hormones and glycemic traits in men and women of the KORA F4/FF4 cohort

T2D-related Traits	P for interaction between 17-OHP & progesterone		
	Men	Women	Women**
Fasting Glucose	-	0.882	0.351
2h-glucose	0.510	-	-
HbA_{1c}	-	-	0.733***
Fasting Insulin	**0.002**	-	-
QUICKI	**0.008**	-	-

All results are from a multivariate linear regression. Interactions between 17-OHP and progesterone were examined by adding a multiplicative term into model 2. Interactions were calculated only for significant associations between exposure and respective outcomes. *Adjusted for baseline age, waist circumference, height, triglycerides, total cholesterol/HDL-cholesterol ratio, hypertension, statin use, smoking, alcohol consumption, physical activity, CRP, eGFR (creatinine-based), TSH, and parental diabetes history. **Sensitivity analyses performed where perimenopausal women (n = outcome specific) were excluded. ***Interaction analysis performed due to significant associations between progesterone and HbA_{1c} upon exclusion of perimenopausal women (n = 66). Significant results are printed in bold. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, SHBG: Sex hormone-binding globulin, TSH: Thyroid-stimulating hormone.*

Supplementary Table 7 - Prospective associations of endogenous progestogens and estrogens with markers of glycemic traits in men of KORA F4/FF4.

		17-OHP	Progesterone	Progesterone[a]	E2	E2[b]	fE2
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Fasting Glucose	**Model 1**	-0.017 (-0.055 – 0.022) P = 0.393	0.021 (-0.015 – 0.056) p = 0.260	-	-0.026 (-0.063 – 0.011) P = 0.174	-	0.016 (-0.025 – 0.057) P = 0.440
	Model 2	-0.019 (-0.057 – 0.020) P = 0.342	0.019 (-0.017 – 0.055) P = 0.302	0.018 (-0.018 – 0.054) P = 0.320	-0.021 (-0.059 – 0.017) P = 0.274	-0.010 (-0.050 – 0.030) P = 0.621	0.014 (-0.028 – 0.056) P = 0.511
2h-glucose	**Model 1**	0.009 (-0.052 – 0.070) P = 0.765	-0.021 (-0.072 – 0.031) P = 0.428	-	-0.011 (-0.070 – 0.047) P = 0.701	-	0.032 (-0.033 – 0.097) P = 0.339
	Model 2	0.013 (-0.048 – 0.074) P = 0.682	-0.021 (-0.073 – 0.031) P = 0.423	-0.021 (-0.073 – 0.031) P = 0.426	-0.005 (-0.064 – 0.055) p = 0.878	0.006 (-0.057 – 0.070) P = 0.841	0.024 (-0.042 – 0.090) P = 0.470
HbA_{1c}	**Model 1**	0.013 (-0.026 – 0.052) P = 0.516	0.007 (-0.029 – 0.043) P = 0.715	-	-0.012 (-0.050 – 0.026) P = 0.542	-	-0.030 (-0.072 – 0.011) P = 0.155
	Model 2	0.007 (-0.032 – 0.045) P = 0.738	0.007 (-0.029 – 0.043) P = 0.703	0.005 (-0.030 – 0.041) P = 0.775	-0.000 (-0.038 – 0.038) P = 0.993	-0.017 (-0.057 – 0.022) P = 0.394	-0.028 (-0.069 – 0.014) P = 0.190
Fasting Insulin	**Model 1**	-0.007 (-0.057 – 0.043) P = 0.785	**0.052 (0.005 – 0.098) P = 0.030**	-	-0.005 (-0.053 – 0.044) P = 0.853	-	0.027 (-0.027 – 0.080) P = 0.327
	Model 2	-0.013 (-0.062 – 0.037) P = 0.617	0.044 (-0.002 – 0.091) P = 0.062	0.043 (-0.003 – 0.090) P = 0.067	0.005 (-0.044 – 0.054) P = 0.835	0.012 (-0.039 – 0.063) P = 0.652	0.020 (-0.033 – 0.074) P = 0.455
QUICKI	**Model 1**	0.009 (-0.042 – 0.059) P = 0.741	**-0.048 (-0.095 – -0.000) P = 0.049**	-	0.006 (-0.044 – 0.055) P = 0.827	-	-0.032 (-0.086 – 0.023) P = 0.256
	Model 2	0.014 (-0.036 – 0.065) P = 0.578	-0.040 (-0.088 – 0.007) P = 0.095	-0.039 (-0.087 – 0.008) P = 0.104	-0.006 (-0.056 – 0.044) P = 0.820	-0.014 (-0.067 – 0.038) P = 0.595	-0.026 (-0.081 – 0.029) P = 0.354

All results are from multivariate linear regression models. Adjusted for baseline values of respective glycemic traits, age, waist circumference, height, triglycerides, total cholesterol/HDL ratio), hypertension, statin use (model 1), smoking, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental diabetes history (model 2). Effect estimates with 95% CIs were calculated for a one sex-specific SD increase on the log scale of progestogen and estrogen levels, respectively. Significant results are printed in bold. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, SHBG: Sex hormone-binding globulin, TSH: Thyroid-stimulating hormone.

[a] *Models were additionally adjusted for albumin.*

[b] *Models were additionally adjusted for SHBG.*

Supplementary Table 8 - Prospective associations of endogenous progestogens and estrogens with glycemic traits in women of KORA F4/FF4.

		17-OHP	Progesterone	Progesterone[a]	E2	E2[b]	fE2
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Fasting Glucose	**Model 1**	0.012 (-0.050 – 0.073) P = 0.713	-0.010 (-0.072 – 0.051) P = 0.742	-	-0.008 (-0.071 – 0.055) P = 0.800	-	0.043 (-0.027 – 0.113) P = 0.230
	Model 2	0.005 (-0.057 – 0.066) P = 0.881	-0.011 (-0.074 – 0.052) P = 0.733	-0.015 (-0.078 – 0.047) P = 0.627	0.004 (-0.059 – 0.067) P = 0.900	0.005 (-0.058 – 0.068) P = 0.872	0.054 (-0.016 – 0.123) P = 0.133
	Model 2 (Sens.)[c]	0.007 (-0.070 – 0.085) P = 0.851	-0.008 (-0.086 – 0.070) P = 0.849	-	0.010 (-0.060 – 0.080) P = 0.784	-	0.054 (-0.016 – 0.123) P = 0.133
2h-glucose	**Model 1**	0.035 (-0.050 – 0.120) P = 0.421	-0.007 (-0.093 – 0.079) p = 0.878	-	-0.060 (-0.146 – 0.025) P = 0.165	-	-0.047 (-0.144 – 0.049) P = 0.336
	Model 2	0.036 (-0.049 – 0.121) P = 0.402	0.004 (-0.083 – 0.091) P = 0.923	0.004 (-0.084 – 0.091) P = 0.935	-0.057 (-0.143 – 0.029) P = 0.192	-0.054 (-0.140 – 0.032) P = 0.219	-0.039 (-0.136 – 0.058) P = 0.433
	Model 2 (Sens.)[c]	0.074 (-0.026 – 0.173) P = 0.147	0.043 (-0.056 – 0.142) P = 0.394	-	-0.029 (-0.118 – 0.061) P = 0.528	-	-0.013 (-0.114 – 0.088) P = 0.800
HbA_{1c}	**Model 1**	0.001 (-0.057 – 0.059) P = 0.970	-0.044 (-0.102 – 0.015) p = 0.141	-	0.019 (-0.041 – 0.078) P = 0.538	-	-0.010 (-0.076 – 0.056) P = 0.761
	Model 2	0.012 (-0.046 – 0.070) P = 0.679	-0.027 (-0.086 – 0.032) P = 0.373	-0.026 (-0.085 – 0.033) P = 0.391	0.024 (-0.035 – 0.083) P = 0.418	0.024 (-0.035 – 0.083) P = 0.423	-0.006 (-0.072 – 0.060) P = 0.853
	Model 2 (Sens.)[c]	-0.004 (-0.076 – 0.067) P = 0.907	-0.025 (-0.097 – 0.046) P = 0.485		0.037 (-0.026 – 0.101) P = 0.248		0.002 (-0.071 – 0.074) P = 0.966
Fasting Insulin	**Model 1**	0.017 (-0.056 – 0.090) P = 0.647	0.010 (-0.063 – 0.083) P = 0.782	-	-0.018 (-0.092 – 0.056) P = 0.637	-	0.024 (-0.059 – 0.107) P = 0.575
	Model 2	0.020 (-0.054 – 0.094) P = 0.589	0.010 (-0.066 – 0.085) P = 0.798	0.007 (-0.068 – 0.083) P = 0.850	-0.016 (-0.091 – 0.059) P = 0.671	-0.020 (-0.096 – 0.056) P = 0.607	0.027 (-0.057 – 0.111) P = 0.525
	Model 2 (Sens.)[c]	0.009 (-0.079 – 0.096) P = 0.848	0.016 (-0.071 – 0.103) P = 0.719		-0.021 (-0.100 – 0.058) P = 0.597		0.022 (-0.066 – 0.111) P = 0.621
QUICKI	**Model 1**	-0.012 (-0.085 – 0.060) P = 0.738	-0.008 (-0.080 – 0.065) P = 0.836	-	0.016 (-0.057 – 0.090) P = 0.667	-	-0.036 (-0.118 – 0.046) P = 0.391
	Model 2	-0.015 (-0.088 – 0.059) P = 0.690	-0.009 (-0.084 – 0.066) P = 0.811	-0.006 (-0.081 – 0.069) P = 0.874	0.012 (-0.063 – 0.087) P = 0.754	0.015 (-0.060 – 0.090) P = 0.693	-0.040 (-0.124 – 0.044) P = 0.348

Model 2 (Sens.)[c]	-0.010 (-0.097 – 0.076) P = 0.812	-0.027 (-0.114 – 0.059) P = 0.533	-	0.012 (-0.066 – 0.091) P = 0.754	-	-0.041 (-0.128 – 0.046) P = 0.356

All results are from multivariate linear regression models. Adjusted for baseline values of respective glycemic traits, age, waist circumference, height, triglycerides, total cholesterol/HDL ratio), hypertension, statin use (model 1), smoking, alcohol consumption, physical activity, CRP, eGFR, TSH, and parental diabetes history (model 2). Effect estimates with 95% CIs were calculated for a one sex-specific SD increase on the log scale of progestogen and estrogen levels, respectively. Significant results are printed in bold. Abbreviations: 17-OHP: 17α-hydroxyprogesterone, CRP: C-reactive protein, eGFR: Estimated glomerular filtration rate, SHBG: Sex hormone-binding globulin, TSH: Thyroid-stimulating Hormone.

[a] *Models were additionally adjusted for albumin.*

[b] *Models were additionally adjusted for SHBG.*

[c] *Sensitivity analyses: Perimenopausal women excluded (n = outcome specific).*

Supplementary Materials and Methods 1 – Steroid Quantification of Hormones in Serum Samples

Steroid quantification in serum was performed at the Metabolomics Platform of the Genome Analysis Center, Helmholtz-Zentrum München. 19 steroids were quantified using an extended version of the Absolute*IDQ*™ Stero17 Kit and LC-ESI-MS/MS: aldosterone, androstenedione (androst-4-en-3,17-dione), androsterone, corticosterone, cortisol, cortisone, 11-deoxycorticosterone, 11-deoxycortisol, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), dihydrotestosterone (DHT), estradiol (E2), estrone, etiocholanolone, 17α-hydroxyprogesterone, progesterone, testosterone, pregnenolone, pregnanediol (the last two steroids were assessed semi-quantitatively). Compound identification and quantification were based on scheduled multiple reaction monitoring measurements (sMRM). Sample preparation and LC-MS/MS measurements were performed as described by the manufacturer in manual UM-STERO17 [1]. Absolute*IDQ*™ Stero17 Kit methodology conforms to the "Guideline on Bioanalytical Method Validation" outlined by the EMEA [2], implying proof of reproducibility within a given error range. Analytical specification for LOD (limit of detection), LLOQ, and ULOQ (lower and upper limit of quantification), specificity, linearity, precision, accuracy, reproducibility, and stability were determined experimentally by Biocrates and are described in the manual AS-STERO17. A detailed method description has been published [3]. Method implementation in the laboratory is described as below:

Serum samples have been prepared in the group of the collaboration partner. All samples have been stored at -80°C until sample preparation for measurements. In the laboratory, 400 µl of ultrapure water were pipetted into each well of a 2 ml 96-well deep well plate. 20 µl of the internal standard mix was added to each well, except the blank cell. Thereafter, 250 µl of blank, calibration standards, quality control samples, and serum samples were pipetted into the distinct respective wells. The well contents were mixed by aspiration using robot-driven pipets. In between, the SPE (solid phase extraction) plate of the kit was conditioned successively with 1 ml of dichloromethane, followed by 1 ml acetonitrile, 1 ml methanol, and 1 ml ultrapure water. Except for sample loading, all SPE purification steps (conditioning, washing, drying, and eluting) were done by pressing solvents through the SPE plate using nitrogen and the positive pressure unit. The velocity was regulated by variation of the nitrogen pressure. After plate conditioning, the mixed samples were loaded onto the SPE plate. The samples dropped through very slowly by gravitation (1-2 drops per second). The SPE plate was washed with 500 µl water, dried for 1 h under nitrogen stream (58 psi). Steroids were subsequently eluted in two steps: 1) Two times with 500 µl

dichloromethane into the same deep well plate (all steroids except DHEAS eluted), the eluate was dried each time for 20 min at 45 psi. 2) With 600 µl acetonitrile into another deep well plate. The first dichloromethane fraction was dissolved in 50 µl of methanol/water (25/75 v/v) and the plate was covered with a lid. To facilitate dissolving, the plate was treated for 1 min in an ultrasonic bath and afterward shaken for 5 min at 600 rpm. The second acetonitrile fraction was diluted with 400 µl of water and after covering the plate was treated like the dichloromethane fraction. Both plates were centrifuged at 50 x *g* and placed into the cooled autosampler (10 °C) for LC-MS/MS measurements. The LC-separation of both fractions was performed using 470 ml ultrapure water and the content of three ampules of the kit as mobile phase A and acetonitrile/methanol/ultrapure water v/v/v 85/10/5 as mobile phase B. Steroids were separated on the HPLC column for Absolute*IDQ*™ Stero17 Kit combined with the precolumn SecurityGuard Cartridge C18 4 x 2 mm (for HPLC, Phenomenex Cat No. AJ0-4286).

HPLC grade solvents were used for sample preparation and measurements. Samples were handled using a Hamilton Microlab STAR™ robot (Hamilton Bonaduz AG, Bonaduz, Switzerland) and a Waters Positive Pressure-96 Processor (Waters GmbH, Eschborn, Germany), besides standard laboratory equipment. Mass spectrometric analyses were using a QTRAP 5500 triple quadrupole system (Sciex Deutschland GmbH, Darmstadt, Germany) equipped with a 1260 Series HPLC (Agilent Technologies Deutschland GmbH, Böblingen, Germany) and a HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland) controlled by the software Analyst 1.6.2. Data evaluation for quantification of metabolite concentrations and quality assessment was performed with the software MultiQuant 3.0.1 (Sciex) and the Met*IDQ*™ software package, which is an integral part of the Absolute*IDQ*™ Stero17 Kit. Metabolite concentrations were calculated using internal standards and reported in nM or ng/ml.

References

[1] BIOCRATES Life Sciences. Absolute*IDQ*™ Stero17 Kit. Increased confidence in steroid hormone analysis. https://biocrates.com/wp-content/uploads/2020/02/Biocrates_Stero17.pdf; 2019. [accessed 6 October 2020]

[2] Committee for Medicinal Products for Human Use (CHMP). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf; 2011. [accessed 6 October 2020]

[3] Breier M, Wahl S, Prehn C, Ferrari U, Sacco V, Weise M, et al. Immediate reduction of serum citrulline but no change of steroid prodile after initiation of metformin in individuals with type 2 diabetes. J Steroid Biochem. 2017; 174;114-9. https://doi.org/10.1016/j.jsbmb.2017.08.004

[4] Rinaldi S, Geay A, Déchaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev. 2002;11:1065-71. https://cebp.aacrjournals.org/content/11/10/1065.long

Supplementary Methods and Materials 2 – Data pre-processing of 17-OHP, Progesterone, and Total E2 measurements

The methods below were used for NA imputation and removal, normalizing the measurements to their plate effects, as well as for calibrating for batch effects for measurements of 17-OHP, progesterone, and total E2, where necessary.

i) NA removal and imputation

NA detection was conducted and removal was performed if more than 40% of steroids of all samples were labeled "NA" or "0" in the raw data set. For the raw data set, only estrone was detected to fulfill these criteria and was therefore subsequently removed from the data set. All other steroids were below 40% NA. These remaining NA or zero values were imputed by a minimum values replacement algorithm, implemented as follows: The minimum value - which could be measured for each metabolite from all plates combined - is gathered. This value is not allowed to be equal to zero as the export from the MetIDQ-System, in which the raw measurement data is translated to quantitative data, unfortunately, uses "NA" and "0" synonymously. To mitigate the effects which could be incurred by using these minimum values directly as imputation values, further steps are to be undertaken.

1. The minimum values are not used directly: they are divided by √2 to emulate their real concentrations being well below the minimally detected ones;
2. These newly calculated values, called “replacers”, are then permuted randomly in a range of 0.75*replacer up to 1.25*replacer to mitigate the statistical effects of repeating the same number over and over again.

ii) Plate normalization

For each plate and metabolite, plate specific mean values (“plate means”) are calculated. For this, the concentrations of each metabolite of the QC-2 samples (n = 5) are used. The plate means are then used to calculate an overall mean of all plates. These steps have to be performed for all metabolites (x_1, x_2, x_3, …, x_n) on all plates (n_1, n_2, n_3, …, n_j) of the data set.

$$Platemean[metabolite(x)] = \frac{\sum C[metabolite(x)]}{N}$$

Where N = number of reference samples and C = concentration.

These plate means are then used to calculate the overall “means of all plates”. This has to be calculated again for all metabolites (x_1, x_2, x_3, …, x_n) for each plate in the data set.

$$Overallmean[X] = \frac{\sum Means[X]}{N}$$

Where X = metabolite (x) of plates ($n…n_j$) and N = number of plates ($n…n_j$)

The plate factors are calculated for each plate separately, e.g. for plate 1 as follows:

$$Factor[Y] = \frac{Overallmean[X]}{Mean[Y]}$$

Where X = metabolite (X) of Plates and Y = Plate(n)[metabolite (X)]

In the final step of normalization, each metabolite concentration is multiplied with their corresponding plate factors, e.g. metabolite concentration of each sample on plate 1 is multiplied with plate factor 1.

$$Normalised[Y] = Factor[Y] \times Concentration[Y]$$

Where Y = Plate(n)[metabolite (X)]

In the last step, the duplicates from Batch 1 of the measurements are removed by averaging them.

ii) Calibration of batch effects of 17-OHP, Progesterone, and E2

Regarding measurements of 17-OHP, progesterone, and E2, serum samples from all KORA F4 participants were initially measured between January and November 2013 (batch 1). Due to measurement problems, they had to be repeated for 980 serum samples with the same methods described above between July 2017 and March 2018 (batch 2). To avoid batch effects, we used 175 duplicate measurements from the same participants of batches 1 and 2 to develop calibration formulas. The intercept and slope of Passing-Bablok regressions were used to calibrate batch 2 measurements with measures from batch 1.

References

[1] BIOCRATES Life Sciences. Absolute*IDQ*™ Stero17 Kit. Increased confidence in steroid hormone analysis. https://biocrates.com/wp-content/uploads/2020/02/Biocrates_Stero17.pdf; 2019. [accessed 6 October 2020]

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Supplementary Methods and Materials 3 – Calculation of Free E2 (fE2)

Free E2 was calculated using mass action equations. The formula is as follows: $[fE2] = [E2] - N - K_aSHBG + \sqrt{(n + [SHBG] - [E2]^2 + (4 \times N \times [E2]) / 2 \times N \times K_aSHBG}$, where [fE2] is the concentration of free E2, [E2] is the concentration of E2, $N = (0.5217 \times ([albumin] \times K_aAlb) + 1)$, [albumin] is the concentration of albumin, K_aAlb is the association constant of E2 to albumin (4.2×10^4 nmol/L), and K_aSHBG is the association constant of E2 to SHBG (3.4×10^8 nmol/L) [4].

References

[1] BIOCRATES Life Sciences. Absolute*IDQ*™ Stero17 Kit. Increased confidence in steroid hormone analysis. https://biocrates.com/wp-content/uploads/2020/02/Biocrates_Stero17.pdf; 2019. [accessed 6 October 2020]

[2] Committee for Medicinal Products for Human Use (CHMP). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf; 2011. [accessed 6 October 2020]

[3] Breier M, Wahl S, Prehn C, Ferrari U, Sacco V, Weise M, et al. Immediate reduction of serum citrulline but no change of steroid prodile after initiation of metformin in individuals with type 2 diabetes. J Steroid Biochem. 2017; 174;114-9. https://doi.org/10.1016/j.jsbmb.2017.08.004

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