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Sex hormone levels and risk of primary open-angle glaucoma in postmenopausal women

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Abstract

Objective—We evaluated the relation of pre-diagnostic sex hormone levels in postmenopausal women with primary open-angle glaucoma (POAG) and intraocular pressure (IOP).

Methods—Among postmenopausal participants of the Nurses' Health Study, POAG cases (n=189; diagnosed 1990–2008) and controls (n=189) were matched on age, fasting status and postmenopausal hormone use at blood draw (1989–1990). Plasma concentrations of estrone sulfate, estradiol, testosterone, sex hormone binding globulin and dehydroepiandrosterone sulfate were assessed. The primary outcome was POAG; in secondary analyses, among cases only, we evaluated maximum untreated IOP at diagnosis. Multivariable-adjusted logistic / multiple linear regression models were used to evaluate tertiles (Ts) of biomarker levels and the two outcomes, adjusting for various potential confounders.

Results—We observed no significant associations of estrone, estradiol, sex hormone binding globulin or dehydroepiandrosterone sulfate with POAG risk or with maximum IOP at glaucoma diagnosis among cases. Suggestive significant associations were observed with highest testosterone and POAG risk (T3 vs. T1 multivariable-adjusted odds ratio:1.84; 95% confidence interval:1.02, 3.33; p-trend:0.10). Similarly, for maximum IOP at diagnosis among cases only (mean 8 years after blood draw), higher testosterone was significantly associated with higher IOP (multivariable-adjusted difference in IOP T3 vs. T1:2.17 mmHg; 95% confidence interval:0.34, 3.99; p-trend:0.02).

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Conclusions—Overall, plasma sex hormone levels in postmenopausal women were not associated with POAG risk; however, a trend of higher testosterone levels being associated with higher POAG risk and higher IOP at diagnosis was observed and needs confirmation.

Keywords

sex hormones; glaucoma; intraocular pressure; menopause

INTRODUCTION

Sex hormones may play a role in the etiology of primary open-angle glaucoma (POAG). Estrogen receptors are present on retinal ganglion cells,¹ and estrogen may have a myriad of effects such as neuroprotection,² enhancing ocular blood flow and lowering intraocular pressure (IOP).³ In glaucoma animal models, exogenous estrogen showed neuroprotective effects.^{4, 5} In human studies, hormone therapy was related to lower POAG risk,^{6, 7} and estrogen metabolism gene variants were collectively associated with POAG in women.⁸ Estrogen influences nitric oxide (NO) signaling, enhancing ocular blood flow and lowering IOP. In support of this, *NOS3* (the gene coding for endothelial NO synthase [eNOS]) variants were associated with POAG defined by IOP > 21 mmHg at diagnosis in women.^{9, 10, 11} Furthermore, a gene–environment interaction was observed whereby postmenopausal hormone (PMH) use modified the relation between *NOS3* variants and POAG risk among postmenopausal women.⁹ In addition, while there is no clear sex predilection in glaucoma incidence, in multiple studies among women, an earlier menopausal transition was related to increased POAG risk, particularly the subtype with IOP > 21 mmHg at diagnosis,^{6, 12, 13} and lower IOP has been reported with the pre-menopausal state and with PMH use.^{3, 14–21} Furthermore, higher body mass index, related to higher circulating estrogen in postmenopausal women,²² has been associated with lower POAG risk.^{23–25} Thus, overall, these data support the possibility of glaucoma-related biological processes being influenced by systemic estrogen levels; estradiol and estrone are the main biologically active estrogens during pre-menopause while estrone is the main circulating estrogen after menopause.²⁶ The role of related biomarkers such as SHBG (sex hormone binding globulin, which binds to estrogens and androgens) and other sex hormones (e.g., androgens of dehydroepiandrosterone sulfate (DHEAS) and testosterone) have been little studied in relation to glaucoma.

While a few studies have evaluated the relation between major circulating sex hormones and IOP,^{27–29} the data is scarce for the relation between sex hormones and glaucoma.³⁰ Given the associations with POAG of PMH use,^{6, 7} early menopause / bilateral oophorectomy,^{6, 12, 13} and higher BMI,^{23–25, 31} which also influence circulating hormone levels in postmenopausal women,^{32–34} we hypothesized that endogenous circulating sex hormone levels may be associated with POAG risk. Using a case-control group nested in the Nurses Health Study (NHS), we evaluated whether sex hormone levels (measured during the preclinical phase for POAG cases, who were age matched to controls) among postmenopausal women is associated with the risk of POAG; secondarily, we evaluated, among cases only, whether sex hormones were associated with maximum untreated IOP at diagnosis that occurred on average 8 years after blood samples were collected.

METHODS

Study sample

NHS,^{35–37} launched in 1976 with 121,700 female registered US nurses, is an ongoing prospective study that follows participants with biennial questionnaires on changes in lifestyle and newly diagnosed medical conditions, including glaucoma; the rate of follow-up has been high at >85% of total possible person-time. Among NHS participants (all females) who were 40+ years old and postmenopausal and provided a blood sample in 1989–1990, 189 POAG cases who were diagnosed from 1998 to 2008 and 189 matched controls were identified. Controls were identified with incidence density sampling³⁸ among those with eye exams, and cases and controls were 1:1 matched on fasting status (9 or more hours since last eat), blood draw date (month and year), age, race/ethnicity and PMH use status as of blood draw. The Partners Healthcare System Institutional Review Board approved this study.

Ascertainment of POAG cases

POAG cases were first identified among NHS participants who self-reported a physician diagnosis of glaucoma on mailed questionnaires sent to participants every two years. To confirm the self-reports of glaucoma, we asked participants information about their diagnosing doctors and for their consent in our seeking confirmatory medical records. All eye care providers of record were requested to send all available visual fields (VFs) and were mailed a supplementary questionnaire to complete and return. A glaucoma specialist (LRP) reviewed the questionnaire (or medical records sent instead of questionnaires) as well as the VFs in a standardized manner. This questionnaire included items about untreated maximum IOP, any secondary causes of high untreated IOP, filtration angle, structural features of the optic nerve, glaucoma surgery, any VF loss and any secondary conditions that may cause VF loss.

Cases we included in analyses had to have at least two reliable VFs (20% for false negative rate and false positive rate and 33% for fixation loss rate) was that showed defects that were reproducible and due to glaucoma, non-occludable angles in both eyes, no secondary causes of IOP elevation (e.g., trauma, uveitis, exfoliation syndrome, pigment dispersion syndrome evident on biomicroscopic anterior segment examinations).

Collection of plasma samples and biomarker measurements

Among NHS participants, 32,826 women provided plasma samples in 1989–1990 by mailing back (26 hours of blood draw) kits for blood collection (the anti-coagulant was heparin). Samples were processed as soon as they were received: they were centrifuged and separated into plasma, red blood cells and buffy coat and stored in freezers.

Case-control sets were assayed together, and samples were ordered randomly within a set, and the laboratory (Mayo Clinic) was masked to case-control status. Samples were assayed for estrogens and testosterone by liquid chromatography-tandem mass spectrometry, for DHEAS by a solid-phase, chemiluminescent immunoassay and for SHBG with competitive immunoassay using electrochemiluminescence detection. Masked replicate quality control samples (10% of the samples) were included in each batch to assess coefficients of variation

(CVs). The CVs showed high reliability: 4.3% for estrone; 7.7% for estradiol; 5.0% for SHGB; 5.7% for testosterone; and 6.6% for DHEAS.

Statistical methods

We used multiple logistic regression models adjusting for matching factors and other covariates to evaluate multivariable-adjusted associations (odds ratios [ORs] and 95% confidence intervals [CIs]) with POAG and plasma markers. We evaluated tertiles of plasma biomarkers, defined using cutpoints based on the biomarker distributions in the controls. As glaucoma is a chronic and insidious disease, covariates were defined as of blood draw using all of the information gathered from 1976 (start of NHS; e.g., body mass index (BMI) was calculated as the average of all BMIs from the 1976 questionnaire to the questionnaire immediately prior to blood collection), except for smoking status, which was the smoking status as of blood collection. We evaluated several nested models incorporating various nested sets of potential confounders: 1) model 1: simple model adjusting for glaucoma family history and major matching factors (age at blood draw, age at blood draw squared, fasting status (<8 hours since last eating, yes or no)); 2) model 2: model 1 with additional adjustment for other possible POAG risk factors (alcohol intake (linear grams/day and the squared term), caffeine intake (linear mg/day and the squared term), physical activity (metabolic equivalents of task-hours per week, as a linear variable and the squared term), body mass index (BMI, as linear variable representing kg/m² and the squared term), smoking status (current, past, never smoker), self-reported physician-diagnosed hypertension, bilateral oophorectomy history and ever PMH use history as of the biennial questionnaire before blood draw; and 3) in secondary analyses to evaluate whether sex hormone levels mediate the association with PMH use, model 3: model 2 with additional adjustment for current PMH use. Race was not adjusted for in models as racial minorities making up <5% of the study population and to prevent model convergence problems. For all biomarkers and linear covariates, we used Rosner's extreme studentized deviate test³⁹ to identify outliers; outliers were truncated such that they were assigned the nearest non-outlying value. We conducted linear tests for trend by evaluating the significance of a variable representing the medians of the tertiles and alternatively evaluated log-transformed continuous variables of sex hormones for maximal power. All significance tests were 2-sided, and the significance level for all analyses was p-value<0.05. The SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

Because associations may be stronger in relation to high-tension subtype of POAG (POAG with highest untreated IOP > 21 mmHg), we also evaluated associations with this subtype. In a case-only analysis, among those with complete IOP data at diagnosis, using multiple linear regression, we evaluated the association between tertiles of hormone levels and differences in maximum untreated IOP at diagnosis. We performed similar nested models as for the analyses for POAG; in addition to the covariates listed above, in these analyses, we also adjusted for the time between blood draw and diagnosis date.

RESULTS

As expected, higher estradiol levels were associated with higher BMI, while higher SHBG levels were associated with lower BMI and lower hypertension prevalence (Table 1). In addition, a history of bilateral oophorectomy was associated with markedly lower levels of testosterone.

Cases generally had similar mean biomarker levels as the matched controls, but they more frequently reported hypertension and glaucoma family history (Table 2). The mean (SD) time from blood sample collection to diagnosis was 8.0 (SD=4.9) years.

Simple models (model 1) and multivariable-adjusted models (model 2) for the biomarkers yielded similar results, indicating minimal influence of covariates (Table 3). Women in the top tertile (T3), compared to women in the bottom tertile (T1) of either estrone or estradiol did not show significant differences in risk of POAG (model 2 for estrone: multivariable OR: 1.51, 95%CI:0.84, 2.70; linear trend p-value [p-trend]:0.12 and for estradiol: OR:1.20, 95%CI:0.67, 2.14; p-trend:0.34) (Table 3). With SHBG and DHEAS, model 2 showed non-significant adverse associations with POAG risk (multivariable OR for the T3 versus T1 was 1.73 (95%CI:0.93, 3.21; p-trend:0.07) with SHBG and 1.39 (95%CI:0.77, 2.51; p-trend: 0.41) with DHEAS). However, highest testosterone levels were significantly associated with higher POAG risk, although the p-trend was not significant (p-trend: 0.10): the multivariable OR for the T2 vs. T1 comparison was 2.03 (95%CI:1.16, 3.56) and for the T3 vs. T1 comparison, the multivariable OR:1.84 (95%CI: 1.02, 3.33). In secondary analyses where we additionally adjusted for current PMH use, there was little change in the results (e.g., testosterone T3 vs. T1 multivariable OR:1.82, 95%CI:1.01, 3.29; p-trend:0.10). Also, in secondary analyses where POAG with highest untreated IOP ≥ 21 mmHg was the outcome, higher testosterone was associated with a significantly increased risk (p-trend:0.04): T3 vs. T1 multivariable OR:2.44 (95%CI:1.19, 5.00). There was no association with other hormones in relation to high-tension glaucoma (p-trends: 0.11). In further secondary analyses (Table 3) where we explored the relative levels of estrogens and testosterone (as the estrogen levels relative to testosterone may be different with PMH use and may be differently associated with POAG than direct estrogen levels), we did not observe that estrogen: testosterone tertile value (tertile value took on values 1, 2 or 3, thus the ratio ranged from 0.33 to 3) was associated with POAG (p-trends: 0.87; Table 3); similarly, the corresponding ratios with testosterone for SHBG and DHEAS were not significant. Finally, we observed no significant interactions between biomarker levels and time between blood draw and diagnosis date of the index case in relation to POAG risk (p-interactions: 0.32).

For the case-only analysis, we had information on maximum untreated IOP at diagnosis among 176 cases (Table 4). In multiple linear regression, except for testosterone levels, there was no association between sex hormones and maximum untreated IOP (p-trends: 0.55). Significantly higher maximum untreated IOP at diagnosis (p-trend: 0.02) was observed with higher testosterone, where the mean IOP difference for the T2 vs. T1 comparison was 1.24 mmHg (95%CI: -0.47, 2.96), and the mean difference for the T3 vs. T1 comparison was 2.17 mmHg (95%CI: 0.34, 3.99).

Because of the non-significant trend of opposite associations between higher estrone and IOP and the adverse association with testosterone, this raised the possibility that the relative concentrations of estrone and testosterone may be associated with IOP differences. Thus, we evaluated the association between the ratio of the tertile values of estrogens to testosterone and max IOP (Table 3). We observed that both higher ratio of the estrone:testosterone tertile values and of the estradiol: testosterone tertile values were associated with lower IOP (estrone: testosterone: T3 vs. T1 difference: -2.53 mmHg; 95%CI: $-4.46, -0.60$; p-trend: 0.01; estradiol: testosterone: T3 vs. T1 difference: -2.31 mmHg; 95%CI: $-4.22, -0.40$; p-trend: 0.01). These similar results likely were because estrone and estradiol were highly correlated (Spearman correlations 0.7). No significant associations were observed with ratios of the tertile values of SHBG:testosterone or DHEAS:testosterone (p-trends: 0.08). Finally, we observed no significant interactions between biomarker levels and time between blood draw and diagnosis date of the index case and differences in max IOP, except for DHEAS, which showed stronger inverse associations with distant IOP measurements (median of 8 years) versus more recent measurements (p-interaction=0.02).

DISCUSSION

Sex hormone levels were not significantly associated with POAG risk in postmenopausal women, except for testosterone, which was associated with significantly adverse associations with POAG, particularly high-tension POAG. Similarly, in secondary analysis among POAG cases, a higher testosterone level was also associated with higher maximum untreated IOP at diagnosis. While the estrogens were not significantly associated with POAG or IOP, a higher estradiol (or estrone) to testosterone ratio, indicating estrogens relative to testosterone, was associated with significantly lower IOP at diagnosis.

There is scarce data on plasma sex hormone levels and glaucoma; one case-control study of 33 women observed that estradiol levels were lower among women <65 years in POAG cases versus controls, although no specific results or statistical test results were provided.³⁰ In our study, we observed no significant associations between estrogens and POAG. Prior studies of the age at menopause^{6, 12, 13, 40} or the use of PMH^{6, 7} that have shown associations with POAG essentially contrasted the state of having near pre-menopausal levels of estrogens versus the dramatically lower levels in menopause. However, we focused on a different study question: whether the much subtler variation in the overall lower postmenopausal estrogen levels (even with a small proportion of women on PMH) was associated with POAG. We recognize that our null findings are not consistent with previous studies suggesting that exposures linked to declining estrogen levels are associated with increased risk of POAG. While our study was powered to detect 2-fold differences in estrogen levels between cases and controls, prior studies suggest that estrogen may have more modest effects on glaucoma risk. For instance, in a study of >152,000 participants, Newman Casey et al.⁷ found that estrogen therapy was associated with a 15% lower POAG risk. Varajanant et al.⁴¹ found that estrogen therapy resulted in a 0.5 mmHg reduction in IOP when post hoc analysis of a randomized clinical trial was performed in the Women's Health Initiative. The sample sizes required to detect these relatively modest effects would need to be extremely large and may make it difficult to confirm that plasma estrogen levels are lower in postmenopausal women with POAG versus appropriate controls.

For the androgens, we observed a significant adverse association between highest testosterone levels and POAG risk and untreated IOP at diagnosis for cases. The association between higher testosterone and higher IOP is consistent with three previous small studies.^{27, 28} Yu et al.²⁷ observed among 23 postmenopausal women not on PMH that testosterone was significantly correlated with higher IOP ($r=0.38$). Similarly, Toker et al.²⁸ observed among both menopausal women on PMH ($n=30$) and menopausal women who never used PMH ($n=32$) that higher testosterone levels were significantly correlated with higher IOP ($r = 0.48$ in women on PMH and $r=0.42$ in women not on PMH) and Karaca Adiyekce et al.²⁹ reported that among 50 women with polycystic ovarian syndrome, higher testosterone was correlated with higher IOP ($r=0.34$). The biological mechanisms underlying this association are unknown, and any proposed mechanism would be speculative. For example, higher testosterone levels could be associated with other factors^{29, 42–44} that may also increase IOP in postmenopausal women, such as higher central corneal thickness (CCT) and higher insulin resistance.^{45, 46} Another major proposed mechanism²⁸ was that in tissues where the enzyme 5 α -reductase is more abundant than the aromatase enzyme, testosterone is preferentially converted by the enzyme 5 α -reductase to dihydrotestosterone, a potent metabolite, which could act directly on androgen receptors⁴⁷ to decrease eNOS (rather than being converted by aromatase to estrogens that may stimulate eNOS activity). For example, in female to male transsexuals given high concentrations of androgen, flow-mediated vasodilatation is low suggesting a negative effect on eNOS-mediated responses⁴⁸. In male pigs going through puberty, increasing plasma testosterone was associated with plasma NO decreases and reductions in endothelium-dependent relaxations of isolated coronary arteries compared to age-matched female⁴⁹, also suggesting an adverse regulatory effect on eNOS. In the eye, any decreased eNOS activity in the aqueous outflow facility would increase IOP; thus, testosterone may antagonize estrogen's ocular hypotensive effects by downregulating eNOS activity. Our observation of significant inverse association with the ratio of the tertile values of estradiol/estrone to that of testosterone (representing being in the highest estrogen category while also being in the lowest testosterone category) provide support for the notion of competing influences on IOP. Indeed, this is also supported by a study⁵⁰ of the PMH Estratest (Solvay, Pharmaceuticals, Inc., Baudette, MN, USA) consisting of estrogen and relatively higher testosterone (2.5 mg of methyltestosterone and 1.25 mg esterified estrogens) in 13 postmenopausal women; it reported increases in IOP (3 mmHg) that were observable after 3 months that were sustained and that IOP returned to baseline levels in the two women in whom treatment was discontinued. Furthermore, while a large de-identified database study⁷ found that estrogen alone was associated with reduced risk of POAG in postmenopausal women, the relation between use of combined estrogen and androgen and POAG was null. Because the relative role of testosterone versus estrogen on IOP or POAG has been little studied, our results point to the need for further studies.

Our study had several limitations. Overall, our sample size was small, and thus, the statistical power was limited to detect modest associations; however, we did observe suggestive associations between highest testosterone and POAG risk and higher IOP among POAG cases. As the study sample only included postmenopausal women who were mainly whites, our study likely has limited generalizability to men or minority populations, in whom the risk of POAG may differ. It is possible that some controls included in our study

might have had glaucoma; however, controls had to have reported an eye exam during the matched case's diagnosis period and given the low overall glaucoma prevalence of <2%,⁵¹ the possible bias would be small and would be in the direction towards the null. In addition, because in postmenopausal women, estrogens and androgens are made via intracrine mechanisms^{52–57} and circulating estrogens or androgens, while reflecting biologically active compounds, do not reflect differences in estrogen or androgen production, our null findings with circulating estrone, estradiol, SHBG and DHEAS levels must be interpreted in this light. Also, future studies using markers of estrogen/androgen production in postmenopausal women, such as estrogen metabolites or glucuronidated metabolites of dihydrotestosterone,^{52–57} would shed more light on the relation between sex steroid levels and glaucoma or IOP in postmenopausal women. Finally, for the IOP analyses, we lacked data on CCT, which may be a mediator of the associations observed; thus, future studies of pre-diagnostic hormone levels of IOP should incorporate measurements of CCT.

Our study's strengths include the extensive matching on age and other factors as well as the exhaustive quality controls measures taken to ensure measurement validity. This study was unique in that it evaluated circulating sex hormones measured during the pre-clinical disease phase. This design minimized bias due to reverse causation that might occur in case-control studies where differences in plasma biomarker levels may be influenced by treatment or disease progression. We also adjusted for multiple covariates that were available at blood draw to minimize confounding bias.

CONCLUSIONS

Sex hormone levels in postmenopausal women were not significantly associated with POAG risk; however, we observed that highest testosterone levels were associated with POAG, and among cases, a higher testosterone level was associated with higher maximum IOP at diagnosis.

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Characteristics of postmenopausal control women (n=189) in the Nurses' Health Study as of blood draw (1989–1990) by extreme tertiles (T) of hormone levels*

Table 1

	Estrone (pg/mL)			Estradiol (pg/mL)			SHBG (nmol/L)			DHEAS (µg/dL)			Testosterone (ng/dL)		
	T1 (n=59)	T2 (n=61)	T3 (n=63)	T1 (n=62)	T2 (n=63)	T3 (n=63)	T1 (n=58)	T2 (n=58)	T3 (n=58)	T1 (n=53)	T2 (n=53)	T3 (n=53)	T1 (n=60)	T2 (n=60)	T3 (n=60)
Biomarker level (geometric mean (SD))	10.6(1.31)	71.9(1.9)	3.1(1.3)	14.0(1.7)	35.6(1.3)	113.9(1.2)	23.6(1.2)	84.6(1.4)	25.7(1.2)	8.6(1.1)	60.9(5.1)	61.4(5.0)	8.6(1.1)	60.9(5.1)	61.4(5.0)
Age (years)	61.5(4.6)	61.0(4.8)	62.7(4.0)	62.7(4.0)	60.4(5.2)	60.9(4.9)	61.4(4.8)	60.6(4.5)	60.6(4.5)	60.6(4.5)	60.6(4.5)	60.6(4.5)	60.6(4.5)	60.6(4.5)	60.6(4.5)
Fasting status, %	29	29	23	27	34	22	32	24	32	24	32	26	32	32	26
Current Smoking, %	9	12	11	10	9	12	4	19	14	4	19	13	14	14	13
Body mass index (kg/m ²)	23.7(3.0)	24.8(4.3)	23.4(2.5)	25.5(4.3)	26.5(4.2)	23.3(3.4)	24.7(4.0)	23.9(3.4)	24.2(3.4)	24.7(4.0)	23.9(3.4)	24.2(3.4)	24.8(3.5)	24.8(3.5)	24.2(3.4)
Family history of glaucoma, %	12	9	9	10	5	10	12	4	11	12	4	11	14	14	11
Hypertension, %	17	27	21	32	41	15	22	31	28	22	31	28	30	30	28
Age at menopause (years)	49.5(4.9)	49.4(3.6)	49.5(4.6)	48.8(4.3)	48.7(4.3)	49.1(4.2)	49.1(4.2)	48.9(4.4)	49.6(3.4)	49.1(4.7)	48.9(4.4)	49.6(3.4)	48.9(5.0)	48.9(5.0)	49.6(3.4)
Bilateral oophorectomy, %	14	25	17	28	17	27	25	15	7	25	15	7	32	32	7
Current postmenopausal hormone use, %	12	80	17	71	12	65	46	27	37	46	27	37	40	40	37
Race/ethnicity [†] : White, %	100	99	100	99	100	98	98	100	100	98	100	100	100	100	100
Non-white, other, %	0	1	0	1	0	2	0	2	0	2	0	0	0	0	0

* Values are means ± SD or percentages and are standardized to the age distribution of the study population.

[†] Race/ethnicity was defined in 5 categories: White, Black, Asian, Latino, Non-white other

Abbreviations: T: tertile; SHBG: sex hormone binding globulin; DHEAS: Dehydroepiandrosterone sulfate

Table 2

Characteristics as of blood draw (1989–1990) in primary open-angle glaucoma cases and controls among postmenopausal Nurses' Health Study participants^{*}

	Case (n=189)	Control (n=189)
Estrone (geometric mean (SD); pg/mL)	25.4(2.5)	25.6(2.4)
Estradiol (geometric mean (SD); pg/mL)	6.3(2.2)	6.3(2.1)
SHBG (geometric mean (SD); nmol/L)	66.7(1.7)	63.8(1.7)
DHEAS (geometric mean (SD); µg/dL)	48.8(1.8)	45.0(1.8)
Testosterone (geometric mean (SD); ng/dL)	15.6(1.6)	14.7(1.6)
Age (years) [†]	61.6(4.8)	61.4(4.8)
Fasting status, % [†]	32	28
Current smoking, %	12	10
Body mass index (kg/m ²)	24.4(3.9)	24.4(3.6)
Family history of glaucoma, %	26	10
Hypertension, %	30	26
Age at menopause (years)	49.3(4.0)	49.3(4.3)
Bilateral oophorectomy, %	19	18
Current postmenopausal hormone use, % [†]	39	39
Race/ethnicity ^{††} : White, %	94	99
Black, %	2	0
Asian, %	1	0
Latino, %	2	0
Non-white, other, %	1	1

^{*} Values are means ± SD or percentages and are standardized to the age distribution of the study population.

[†] Matching factors

^{††} Race was defined in 5 categories: White, Black, Asian, Latino, Other

Abbreviations: SHBG: sex hormone binding globulin; DHEAS: Dehydroepiandrosterone sulfate

Table 3

The association between hormone tertiles (T; 1989–1990) and incident primary open-angle glaucoma (1990–2008): odds ratios (95% Confidence Intervals)

Biomarker	Case N/Total N/ [Range]	Odds Ratio (95% CI)	p-trend ^a	Ratio of Tertiles of Biomarkers ^b	Odds Ratio (95% CI)	p-trend ^a
Estrone: (pg/mL)	Model1 ^c			(Case N/ Total N) Model 1 ^c		
	T1	60/119 [5.0–15.0]	1.00 (reference)	Testosterone tertile ratio ^b	1.00 (reference)	
	T2	58/121 [16.0–28.0]	0.94 (0.56, 1.58)		0.84 (0.51, 1.39)	
	T3	67/128 [29.0–200.0]	1.13 (0.67, 1.89)		0.86 (0.49, 1.48)	
	Model2 ^d			Model 2 ^d		
	T1		1.00 (reference)	T1	1.00 (reference)	
	T2		1.00 (0.58, 1.73)	T2	0.92 (0.55, 1.56)	
	T3		1.51 (0.84, 2.70)	T3	0.95 (0.51, 1.76)	0.90
			0.12			
Estradiol: (pg/mL)/T1	Model1			Estradiol: Testosterone tertile ratio ^b		
	T1	64/126 [1.8–4.2]	1.00 (reference)	Model 1	1.00(reference)	
	T2	55/115 [4.3–7.6]	0.79(0.46, 1.33)	T1(63/124)	0.97 (0.59, 1.59)	
	T3	67/130 [7.7–75.0]	1.01 (0.60, 1.68)	T2(75/147)	0.90 (0.52, 1.55)	
	Model2			T3(48/100)		
	T1		1.00 (reference)	Model 2		
	T2		0.79 (0.45, 1.36)	T1	1.00(reference)	
	T3		1.20 (0.67, 2.14)	T2	1.00 (0.59, 1.69)	
			0.34	T3	0.96(0.52, 1.77)	0.87
SHBG (nmol/L)	Model1			SHBG: Testosterone tertile ratio ^b		
	T1	51/109 [18.0–51.7]	1.00 (reference)	Model 1	1.00 (reference)	
	T2	56/114 [51.8–82.8]	1.14 (0.66, 1.95)	T1 (56/108)	0.85 (0.50, 1.45)	
	T3	71/129 [82.9–144.0]	1.45 (0.85, 2.46)	T2(59/125)	1.08(0.63, 1.86)	
	Model2			T3(63/119)		
	T1		1.00(reference)	Model2	1.00(reference)	
	T2		1.19(0.66, 2.16)	T1	0.91(0.52, 1.60)	
			0.07	T2		

Biomarker	Case N/Total N/ [Range]	Odds Ratio (95% CI)	p-trend ^a	Ratio of Tertiles of Biomarkers ^b	Odds Ratio (95% CI)	p-trend ^a
DHEAS (µg/dL)	T3	1.73 (0.93, 3.21)		DHEAS:	1.17 (0.63, 2.19)	0.53
	Model1			Testosterone		
	T1	1.00(reference)		tertile ratio ^b	1.00 (reference)	
	T2	1.55(0.88,2.72)		T1 (54/106)	1.19(0.70,2.00)	
	T3	1.44 (0.82, 2.54)		T2 (75/141)	0.99 (0.54, 1.81)	
	Model2			T3 (39/ 80)		
Testosterone (ng/dL)	Model1		0.41	Model 2		0.64
	T1	1.00 (reference)		T1	1.00 (reference)	
	T2	1.58 (0.88, 2.83)		T2	1.19 (0.68, 2.07)	
	T3	1.39 (0.77, 2.51)		T3	0.90 (0.47, 1.71)	
	Model1					
	T1	1.00(reference)				
	T2	1.78 (1.05,3.00)	0.10			
	T3	1.59(0.92, 2.75)				
	Model2					
	T1	1.00 (reference)				
	T2	2.03 (1.16, 3.56)				
	T3	1.84(1.02, 3.33)				

Abbreviations: SHBG: sex hormone binding globulin; DHEAS: Dehydroepiandrosterone sulfate; T: tertile

^a p-trends were based on the significance of variable taking on the median value of each tertile. Alternative p-values based on linear log transformed hormones were as follows: 0.36 for estrone, 0.39 for estradiol, 0.08 for SHBG, 0.17 for DHEAS and 0.13 for testosterone.

^b Ratio of the tertile values of two biomarkers (tertile values are 1, 2, 3 so the ratio ranges from 0.3333 (=1/3) to 3 (=3/1)).

^c Model 1 adjusted for age at blood draw, age at blood draw squared term, fasting status (<8 hours since last ate, yes or no)), family history of glaucoma

^d Model 2=Model 1+ alcohol intake (g/day), alcohol intake squared term, caffeine intake (mg/day), caffeine intake squared term, physical activity (metabolic equivalents of task-hours/week), physical activity squared term, smoking status (current / past / never), hypertension, body mass index (kg/m2), body mass index squared term, bilateral oophorectomy (yes or no), ever PMH use history as of the biennial questionnaire before blood draw.

Table 4

Among incident POAG cases only (n=176), the association between hormone tertiles (T) as of 1989–1990 and maximum untreated intraocular pressure (IOP) as differences in IOP (95% Confidence Intervals)^a.

Biomarker	N/ [Range]	Difference in Max IOP (95% CI)	p-trend ^b	Ratio of Tertiles of biomarkers ^c	Difference in Max IOP (95% CI)	p-trend ^b
Estrone: (pg/mL)	Model1 ^d			Estrone: Testosterone tertile ratio ^c		
	T1	55 [5.0–15.0]	0.00 (reference)	Model 1 ^d T1 (n=59)	0.00 (reference)	
	T2	54 [16.0–28.0]	–0.70 (–2.30, 0.91)	T2 (n=70)	–0.43 (–1.89, 1.03)	
	T3	64 [29.0–200.0]	–0.12 (–1.66, 1.43)	T3 (n=44)	–1.61 (–3.27, 0.05)	
	Model2 ^e			Model 2 ^e		
	T1		0.00 (reference)	T1	0.00 (reference)	
	T2		–0.77 (–2.50, 0.96)	T2	–0.84 (–2.38, 0.70)	
	T3		–0.22 (–2.04, 1.60)	T3	–2.53 (–4.46, –0.60)	0.01
			0.93			
Estradiol: (pg/mL)	Model1			Estradiol: Testosterone tertile ratio ^c		
	T1	57 [1.8–4.2]	0.00 (reference)	Model 1 T1 (n=58)	0.00 (reference)	
	T2	52 [4.3–7.6]	0.51 (–1.13, 2.15)	T2 (n=70)	0.14 (–1.33, 1.61)	
	T3	64 [7.7–75.0]	0.15 (–1.41, 1.71)	T3 (n=45)	–1.63 (–3.29, 0.04)	
	Model2			Model 2		
	T1		0.00 (reference)	T1	0.00 (reference)	
	T2		0.42 (–1.31, 2.15)	T2	–0.46 (–2.06, 1.14)	
	T3		0.32 (–1.48, 2.13)	T3	–2.31 (–4.22, –0.40)	0.01
			0.81			
SHBG (nmol/L)				SHBG: Testosterone tertile ratio ^c		
	Model1			Model 1 T1 (n=49)	0.00 (reference)	
	T1	47 [18.0–51.7]	0.00 (reference)	T1 (n=49)	0.00 (reference)	
	T2	51 [51.8–82.8]	0.57 (–1.15, 2.28)	T2 (n=59)	0.07 (–1.57, 1.71)	
	T3	68 [82.9–144.0]	0.35 (–1.26, 1.97)	T3 (n=58)	–1.11 (–2.80, 0.57)	
	Model2			Model 2		
	T1		0.00 (reference)	T1	0.00 (reference)	

Biomarker	N/ [Range]	Difference in Max IOP (95% CI)	p-trend ^b	Ratio of Tertiles of biomarkers ^c	Difference in Max IOP (95% CI)	p-trend ^b
DHEAS (µg/dL)	T2	0.86 (−1.00, 2.71)	0.60	DHEAS: Testosterone tertile ratio ^c	−0.30 (−2.06, 1.46)	0.08
	T3	0.70 (−1.21, 2.61)			−1.78 (−3.89, 0.33)	
	Model1					
	T1 (n=47)	0.00 (reference)			0.00 (reference)	
	T2 (n=73)	−1.61 (−3.23, 0.01)			−0.71 (−2.20, 0.77)	
	T3 (n=35)	−0.09 (−1.74, 1.56)			−1.31 (−3.12, 0.51)	
Testosterone (ng/dL) T1	Model2		0.55			0.20
	T1	0.00 (reference)			0.00 (reference)	
	T2	−1.43 (−3.09, 0.23)			−0.65 (−2.16, 0.86)	
	T3	0.01 (−1.65, 1.68)			−1.26 (−3.14, 0.62)	
	Model1					
	T1	0.00 (reference)				
	T2	0.74 (−0.90, 2.39)				
	T3	1.63 (−0.08, 3.33)				
	Model2					
	T1	0.00 (reference)				
	T2	1.24 (−0.47, 2.96)				
	T3	2.17 (0.34, 3.99)				

Abbreviations: SHBG: sex hormone binding globulin; DHEAS: Dehydroepiandrosterone sulfate

^aCases (n=13) with missing maximum IOP were excluded.

^bp-trends were based on the significance of variable taking on the median value of each tertile. Alternative p-values based on linear log transformed hormones were as follows: 0.92 for estrone, 0.92 for estradiol, 0.87 for SHBG, 0.83 for DHEAS and 0.07 for testosterone.

^cRatio of the tertile values of two biomarkers (tertile values are 1, 2, 3 so the ratio ranges from 0.3333 (=1/3) to 3 (=3/1)).

^dModel 1 adjusted for time between blood draw to diagnosis, age at blood draw, age at blood draw squared term, fasting status (<8 hours since last ate, yes / no), family history of glaucoma

^eModel 2=Model 1+ smoking status (current / past / never), alcohol intake (g/day), alcohol intake squared term, caffeine intake (mg/day), caffeine intake squared term, physical activity (metabolic equivalents of task -hours/week), physical activity squared term, hypertension, body mass index (kg/m²), body mass index squared term, bilateral oophorectomy (yes or no), ever PMH use history as of the biennial questionnaire before blood draw.