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Association of urinary sex steroid hormones with urinary calcium, oxalate and citrate excretion in kidney stone formers

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Running Head:

Sex hormones and urinary lithogenic risk factors

ABSTRACT

Background. Sex-specific differences in nephrolithiasis with respect to both distribution of prevalence and stone composition are widely described and may be influenced by sex hormones.

Methods. We conducted a cross-sectional analysis of the relationship between 24-hour urinary sex hormone metabolites measured by gas chromatography–mass spectrometry with urinary calcium, oxalate and citrate excretion in a cohort of 628 kidney stone formers from a tertiary care hospital in Switzerland, taking demographic characteristics, kidney function and dietary factors into account.

Results. We observed a positive association of urinary calcium with urinary testosterone and 17β-estradiol. Positive associations of urinary calcium with dehydroepiandrosterone, 5α-DH-testosterone, etiocholanolone, androsterone, and estriol were modified by net gastrointestinal alkali absorption or urinary sulfate excretion. As the only sex hormone, dehydroepiandrosterone was inversely associated with urinary oxalate excretion in adjusted analyses. Urinary citrate correlated positively with urinary testosterone. Associations of urinary citrate with urinary androsterone, 17β-estradiol and estriol were modified by urinary sulfate or sodium, or by sex.

Conclusions. Urinary androgens and estrogens are significantly associated with urinary calcium and citrate excretion, and associations are in part modified by diet. Our data furthermore reveal dehydroepiandrosterone as a novel factor associated with urinary oxalate excretion in humans.

What is already known about this subject

Little is known about the influence of sex hormones on lithogenic risk factors in humans and available data are conflicting. This may at least partially be due to limitations of analytical methods used for sex steroid hormone analyses in the past including immunoassays that are known for lower specificity compared to chromatography-mass spectrometry techniques. Also, blood levels of steroid hormones depend on the timing of blood sampling, thus, they likely not comprehensively represent daily production of sex steroid hormones in kidney stone formers.

What this study adds

Here we quantified twelve urinary sex steroid hormones in 24-hour urines using gas chromatography–mass spectrometry in a well-characterized large cohort of 628 kidney stone formers and assessed the association with urinary calcium, oxalate and citrate excretion. Our data demonstrate that androgens and estrogens are predictors of urinary calcium and citrate excretion in humans and may exert both stone promoting and stone inhibitory effects, and some of these effects seem to be influenceable by dietary habits. Our data furthermore reveal dehydroepiandrosterone as a novel factor associated with urinary oxalate excretion in humans.

What impact this may have on practice or policy

These results hint at an interplay between sex hormones and dietary factors on urine lithogenicity and may encourage further research efforts in this direction. Determining urinary sex steroid hormones together with dietary markers may improve future risk assessment in kidney stone formers. Additionally, it may encourage to place further emphasis on dietary advices and guidance as modifiable factors in clinical practice.

Keywords: sex steroid hormones, nephrolithiasis, lithogenic risk factors, dietary factors

INTRODUCTION

Nephrolithiasis has been described in medical reports across different countries and cultures over thousands of years, and it remains a prevalent and recurrent disorder causing substantial global burden of disease [1, 2]. Sex-specific differences in nephrolithiasis with respect to both distribution of prevalence and stone composition may be explained by varying interplay of genetic and environmental factors [3-6], however, genetic risk factors may be less important in women than in men as recently suggested by a twin study [7].

Sex hormones may influence stone formation and some previous research aimed at this direction. In male kidney stones formers, higher blood levels of sex hormones and a more intensive staining of the androgen receptor in the nuclei of distal tubule epithelial cells in the kidney were found compared to healthy controls [8-10]. In other studies, however, no alterations of sex hormone blood levels or lower levels of urinary testosterone were observed in kidney stone formers [11-13]. In two randomized placebo-controlled trials, estrogen replacement therapy (ERT) increased the risk to develop kidney stones in healthy postmenopausal women [14], however, a recent meta-analysis did not support a link between post-menopausal hormone levels and the risk of kidney stone formation [15].

The driving force for calcium oxalate stone formation is calcium oxalate supersaturation, and its most important predictors are urinary volume and urinary concentrations of calcium, oxalate and citrate [16-19]. The potential impact of menopause and ERT on 24-hour excretion of these urinary solutes has been previously investigated. In postmenopausal women no differences were found compared to non-menopausal controls after multivariable adjustment [20]. ERT was associated with higher excretion of citrate and calcium, but not with oxalate, in postmenopausal kidney stone formers in one study [21], and with lower 24-hour and 2-hour fasting excretion of calcium in another study [22].

Thus, previous studies yielded conflicting results with respect to the association of sex hormones with stone formation rate or urinary lithogenic risk factors. This may be at least partially due to methodological reasons. Different analytical methods were used for sex steroid hormone analyses in the past, among them immunoassays, that are known for lower specificity compared to chromatography-mass spectrometry techniques [23]. In addition, blood levels of steroid hormones depend on the timing of blood sampling, thus, they likely not comprehensively represent the daily production of sex steroid hormones.

Specific objectives of this study were to assess in 24-hour urine samples of kidney stone formers the relationship of urinary sex hormones and their metabolites, measured by gas chromatography–mass spectrometry (GC-MS), with urinary calcium, oxalate and citrate excretion.

MATERIALS AND METHODS

Bern Kidney Stone Registry participants were recruited at the Division of Nephrology and Hypertension at the Bern University Hospital, Bern, Switzerland and underwent a comprehensive metabolic workup including 24-hour urinary steroid hormone profiling [24, 25]. The Bern Kidney Stone Registry adheres to the Declaration of Helsinki and was approved by the Ethical Committee of the Kanton Bern (# 95/06). All participants provided written informed consent. Kidney stone patients at least 18 years of age, suffering from at least one stone episode and with an available urinary steroid hormone profile, in total 628 patients, were eligible for analysis. Participants with characteristics that may strongly influence calciuria, oxaluria and / or citraturia were excluded from analysis: history of primary hyperparathyroidism (n=16), a total plasma calcium >2.5mmol/L with parathyroid hormone (PTH) >65 pg/mL (n=20), sarcoidosis (n=3), pregnancy or lactation during the study visit (n=2), anorexia nervosa or bulimia (n=3), active malignant diseases (n=15), solid organ transplantation (n=2), the intake

of phosphate supplementation (n=1), bisphosphonates (n=11), cinacalcet (n=1), denusomab (n=1), teriparatide (n=1), loop diuretics (n=10), potassium-sparing diuretics (n=9), or carboanhydrase inhibitors (n=1). Additionally, participants with conditions interacting with steroid hormone metabolism were excluded from analysis: diagnosis of polycystic ovary syndrome (n=1), history of unilateral or bilateral oophorectomy (n=1) or orchiectomy (n=1), use of the following fix or on-demand medication: topic or systemic glucocorticoids (n=24), hormones used for menopause treatment, to prevent conception or to suppress menstrual bleeding (n=32), use of anabolic steroids (n=1), anti-androgen or anti-estrogen therapy (n=7), antiepileptic agents (n=3). Finally, 126 patients were excluded from analysis.

Laboratory measurements and definitions

The urinary steroid profile consists of 40 steroid hormones and metabolites, including 12 sex steroid hormones and metabolites, and was quantified in a 24-hour urine collection on a random outpatient diet by an *in-house* adapted GC-MS method in the steroid laboratory of the Department of Nephrology and Hypertension at the Bern University Hospital, Switzerland [26, 27]. Established steroid hormone metabolite ratios reflecting steroidogenic enzyme activities towards sex steroid hormone biosynthesis (**Figure 1)** were calculated from steroid hormone compounds [28, 29]. All other urinary parameters were measured centrally by standard clinical laboratory methods from two 24-hour urine collections on two consecutive days. Urine collections with a creatinine excretion value outside a predefined 2.5^{th}-97.5^{th} confidence interval based on urinary creatinine excretion values from the adult Swiss population were regarded as under- or overcollected and were therefore excluded from the analysis [30]. Mean 24-hour excretion values from two urine collections, if available, were used for statistical analysis. The 24-hour urinary sodium excretion was used to assess salt intake. Net gastrointestinal alkali absorption (NGIA) was calculated from 24-hour urinary electrolytes [31]. The 24-hour urinary sulfate excretion was used as a marker of dietary acid ash content from

sulfur-containing amino acids. Fourier transform infrared spectroscopy was performed for kidney stone analysis.

Statistical analysis

Extreme outliers of each steroid hormone metabolite were defined as outside the range of the 25th percentile minus 3×interquartile range to the 75th percentile plus 3×interquartile range and were also excluded from analysis. A descriptive statistic was created, which included the available data for each variable of interest for the whole study population and also dichotomized by sex. Sex-specific differences in urinary excretion of sex hormone metabolites, calcium, oxalate and citrate were assessed by Wilcoxon rank-sum test. Type and strength of relationship between two sex hormone metabolites was explored by visual inspection of bivariate scatterplots and by calculation of bivariate Spearman's and Pearson's correlation. The potential influence of sex steroid hormone metabolites as main predictor variables on the 24-hour excretion of the three lithogenic solutes calcium, oxalate and citrate as outcome variables was assessed by univariate and adjusted linear regression analyses. To fit linear model assumptions, the outcome variables calcium and citrate were square root–transformed and the outcome variable oxalate was log-normal transformed. All continuous predictor variables and all outcome variables were scaled for regression analyses. Adjusted analyses included the seven co-variables sex, age, body mass index (BMI), eGFR, NGIA, sulfate and sodium as potential confounders. To account for potential effect modifications, significant interaction terms between the main predictor variables and the co-variables were added to adjusted regression models by forward selection analyses. Adjusted beta coefficients and corresponding 95% confidence intervals were calculated and reported. A P value <0.05 was considered to be significant. Models were validated by graphical analysis for homogeneity of variance,

normality of residuals, and highly influential observations. All final adjusted models were further tested for their robustness by restricting the adjusted analysis to a subset of the study population without the following additional potential confounders: intake of over-the-counter or prescribed calcium and/or vitamin D supplements (n=17), thiazide diuretics (n=38), potassium or magnesium citrate or sodium bicarbonate (n=21) and postmenopausal status (n=39 out of n=100 women). Interaction terms that did not persist robustly in this sensitivity analysis were omitted from the final adjusted models. All statistical analyses were performed with R software, version 3.5.0 (R Foundation for Statistical Computing).

RESULTS

Characteristics of the study population

Characteristics of 502 kidney stone formers included in the final analysis are shown in **Table 1**. Age of study participants ranged from 18 to 80 years, and the percentage of female stone formers was 20.3% (N=103), among them 38.8% (N=40) self-reported post-menopausal women. Urinary sex steroid metabolites exhibited a right-skewed distribution and median values of each sex group were within the range of reference intervals recently published for men and women [32]. The excreted amount of all ten androgen metabolites was substantially higher in men than in women across all age groups, whereas no sex specific differences were found for the excretion of the two estrogen metabolites 17β-estradiol and estriol. Kidney stone analysis was available in 80.9% (N=406) of patients, and most of the stones contained calcium oxalate (92%). Higher 24-hour excretions in men than in women were found for calcium, oxalate and citrate. Correlation analyses between sex hormone metabolites revealed a strong positive linear relationship of dehydroepiandrosterone (DHEA) with its two direct metabolites androstenediol and 16α-OH-DHEA, a moderate positive relationship of testosterone and 17β-estradiol with their direct metabolites 5α-DH-testosterone and estriol, respectively, and

throughout weak positive relationships between androgen metabolites and estrogen metabolites (**Supplementary Table S1**).

Association analysis with calcium excretion as outcome variable

In unadjusted analyses, a positive association of urinary calcium excretion was found with urinary excretion of all ten androgen metabolites and with 17β-estradiol, but not with estriol **(Table 2)**. After multivariable adjustment **(Table 2 and Figures 2A-2P)**, the association with testosterone, 5α-androstanediol, 11β-OH-androsterone, and 17β-estradiol remained robust. For the association with urinary calcium excretion, significant interactions with the dietary markers NGIA or sulfate were found for the most abundant circulating androgen metabolite DHEA, for the most potent androgen 5α-DH-testosterone, for the most abundant urinary androgen metabolites androsterone and etiocholanolone, for estriol, and for hormone metabolite ratios. The described interaction effects remained robust in sensitivity analyses **(Supplementary Table S2).**

Significant positive associations were found with the co-variables eGFR (range of β coefficients: 0.21-0.25, range of *p*-values: 2.2×10^{-4}-8.9×10^{-6}), with urinary excretion of sodium (β: 0.11-0.16, *p*: 0.028-0.0017) and sulfate (β: 0.23-0.26, *p*: 2.0×10^{-8}-4.6×10^{-7}), and a significantly lower urinary calcium excretion was found in women compared to men (β: -0.50 to -0.33, *p*: 1.1×10^{-5}-0.0078).

Association analysis with urinary oxalate excretion as outcome variable

In unadjusted analyses, positive associations of urinary oxalate excretion were found with the urinary excretion of six androgen metabolites **(Table 3)**, including testosterone. In multivariable analyses **(Table 3 and Figures 3A-3H)**, associations with androgen metabolites

including 5α-DH-testosterone did not remain significant. The association with DHEA became significantly negative. No significant association was found with the estrogen metabolites 17β-estradiol and estriol. For steroidogenic enzyme activities, significant interactions were found for the 17α-hydroxylase activity of the combined Δ^4- and Δ^5-pathways with sulfate excretion and for the aromatase activity with age. Interaction effects were robust in sensitivity analyses **(Supplementary Table S3).**

With regard to co-variables in regression models, a significant lower oxalate excretion was found in women compared to men (β-range: -0.60 to -0.47, *p*-range: 3.4×10^{-6}-$5.3\times^{-4}$) and a significant positive association was found with urinary excretion of sodium (β-range: 0.18-0.23, *p*- range: 9.5×10^{-5}-5.6×10^{-4}).

Association analysis with urinary citrate excretion as outcome variable

In unadjusted analyses, a positive association of urinary citrate excretion was found with the three androgen metabolites testosterone, 5α-DH-testosterone, and 11β-OH-androsterone and with both estrogen metabolites 17β-estradiol and estriol **(Table 4)**. In multivariable adjustment **(Table 4 and Figures 4A-4L)**, the association with testosterone remained robust and the positive association with 16α-OH-DHEA became significant. For the association with urinary citrate, significant interactions were found with the dietary markers sulfate for androsterone, with sodium for both estrogen metabolites, and with NGIA for hormone metabolite ratios. Interaction effects were robust in sensitivity analyses **(Supplementary Table S4)**.

The assessment of co-variables revealed a significant positive associations with age (β-range: 0.29-0.38, range of *p*-range: 3.0×10^{-8}-1.7×10^{-5}), BMI (β-range: 0.095-0.13, *p*-range: 0.040-0.0051), eGFR (β-range: 0.27-0.33, *p*-range: 9.6×10^{-8}-1.0×10^{-5}), NGIA (β-range: 0.25-0.33, *p*-range: 2.4×10^{-11}-8.2×10^{-8}), and sulfate (β-range: 0.093-0.11, *p*-range: 0.021-0.048), and a

significant lower citrate excretion was found in women compared to men (β-range: -0.27 to -0.36, *p*-range: 0.028-0.0044).

DISCUSSION

To our knowledge, this is the first study within the area of kidney stone research that considers such a rich phenotype of steroid hormone metabolism in relation to the most important predictive solutes of calcium oxalate supersaturation as the driving force for calcium stone formation. Our results reveal an association of urinary androgens on urinary calcium excretion. This may be due to a direct effect of androgens on renal calcium transporters, as previously reported in mice [33]. Compared to sham-operated controls, orchidectomized mice displayed reduced urinary calcium excretion accompanied by upregulated mRNA and protein levels of the luminal transient receptor potential vanilloid-subtype 5 channel (TRPV5) and of the intracellular calcium-binding protein calbindin-D_{28K} in the distal convoluted tubule (DCT). Treatment of orchidectomized mice with testosterone normalized both urinary calcium excretion and the expression of TRPV5 and calbindin-D_{28K}. Serum levels of calcium, parathyroid hormone, 1,25-dihydroxyvitamin D_3, and estrogen did not differ between treatment groups, suggesting a direct effect of testosterone on renal calcium handling. This notion is further supported by *in vitro* experiments – treatment with 5α-DH-testosterone led to an upregulation of TRPV5 in primary tubular cells [33]. Sex steroid hormone receptors are expressed in the kidney of women and men [34]. In addition, the enzymes 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase/17,20-lyase, P450 oxidoreductase, 5α-reductase, and aromatase, which are all essential for sex steroid hormone biosynthesis, are expressed in the kidney [35-37]. Therefore, the additional presence of an extragonadal sex steroid hormone

metabolism in the human kidney appears to be very probable. Beside a direct action on the kidney, however, sex steroids may also influence renal calcium excretion indirectly e.g. by affecting calcium metabolism in gut or bone.

Adjusted analyses in our study revealed positive associations between urinary calcium excretion with DHEA at a lower NGIA level and at a higher urinary sulfate level, indicating that the association is highly dependent on dietary alkali (high NGIA, low sulfate) and acid (low NGIA, high sulfate) intake. In addition, positive associations between urinary calcium with 5α-DH-testosterone and etiocholanolone at a lower NGIA level and with androsterone at a lower sulfate level were found. Thus, apparent contrary results were obtained for the interaction effects between sulfate and DHEA and sulfate and androsterone.

Positive associations of urinary oxalate with androgen metabolites observed in unadjusted analysis did not remain significant after adjustment. Addition of the co-variable sex to univariable regression models caused the largest change in the association between androgen metabolites and oxalate excretion. Thus, sex was the key confounding variable in the models. In a group of 48 men from India with calcium nephrolithiasis, 24-hour urinary oxalate correlated weakly with free serum testosterone (Pearson's r=0.297, *p*-value=0.040) [38]. No adjusted analyses were performed in this study, however, likely due to the small number of study participants. Our results demonstrate that even strong associations between androgens and oxalate excretion in unadjusted analyses may not be robust to multivariable adjustment due to strong confounders.

In contrast, DHEA and its main metabolite 16α-OH-DHEA showed a negative association with oxalate, and for DHEA this association became significant in adjusted analyses. Together with its sulfonated form, DHEA is the most abundant circulating steroid hormone exhibiting weak androgenic activity, but its physiological role in brain and liver exceeds those of a simple sex steroid precursor and comprises numerous signaling pathways [39]. Endogenously produced

oxalate derives from glyoxalate. Glyoxalate is formed in the liver from glycolate by glycolate oxidase (GO) and can be converted to glycolate by glyoxylate/hydroxypyruvate reductase (GRHPR) or to glycine by alanine-glyoxylate aminotransferase (AGXT) [40]. DHEA-treated castrated rats had higher hepatic enzyme activities of GRHPR and AGXT [41]. Hence, DHEA may directly modulate endogenous oxalate production in the liver.

We also observed a positive association of urinary citrate, a major inhibitor of calcium kidney stone formation, with testosterone and with androsterone at a low urinary sulfate excretion. This finding is in line with results from patients with polycystic ovary syndrome (PCOS) with high free serum testosterone which had higher 24-hour urinary citrate excretion compared to the subgroup with low free serum testosterone [42]. The authors of this study speculated that higher citraturia may have been induced by metabolic alkalosis due to increased mineralocorticoid activity with PCOS. To test this in our study, we added plasma bicarbonate as an additional co-variable to adjusted analysis of the association between urinary testosterone and citrate, however, the association remained essentially unchanged, indicating that plasma bicarbonate was not an additional confounding factor nor an effect modifier for the association. Physiologically, an increase of urinary citrate excretion makes absolute sense in a situation when urinary calcium excretion rises. The parallel increase of urinary citrate and calcium associated with higher urinary androgens mitigates the risk to develop kidney stones or nephrocalcinosis. While the mechanisms underlying the effect of androgens on tubular calcium handling have been studied, as outlined above, the impact of sex hormones on citrate transport in the proximal tubule have not been studied to our knowledge. As is the case for calcium, androgens may not only influence renal citrate reclamation in the proximal tubule but also alter circulating citrate levels (i.e. filtered load).

In contrast to our results, no sex differences for the 24-hour excretion of urinary citrate were found in a Chicago cohort of 330 calcium oxalate stone formers [43] or in 807 kidney stone

formers derived from three different US American cohorts of health professionals [44]. In our Bern cohort, higher 24-hour citrate excretion rates in men compared to women were found in both unadjusted and adjusted analyses, thus, apparent differences are not an effect of statistical methods. The 24-hour urine collections in the Bern cohort contain similar amounts of creatinine compared to the US cohorts, arguing against a systematic under- or over-collections of urine samples in one of the cohorts. Differences in urinary citrate excretion compared to the Chicago cohort may indicate different sex specific dietary habits in the rural population of Bern, and these sex specific differences may blur with an increasing degree of urbanization or even may disappear in metropolitan regions like Chicago. Furthermore, mean citrate excretion values in the US American cohorts of health professionals were considerably higher in both sexes compared to the cohorts from Chicago and Bern and may indicate a healthier, less meat-based diet in health professionals, or simply a better kidney function in the group of health professionals.

In contrast to urinary androgens, the situation is more complex with urinary estrogens. Our results suggest that urinary calcium excretion is positively associated with urinary 17β-estradiol and also with estriol in the setting of low urinary sulfate excretion (i.e. low dietary acid intake), whereas urinary oxalate excretion is not associated with any urinary estrogen. The association of urinary citrate excretion with estrogens is also diet dependent, being positive with 17β-estradiol (men and woman) and estriol (men only) in the setting of low urinary sodium excretion (i.e. low sodium intake or higher extrarenal sodium loss) and positive with estriol at a low sodium intake and in men only. 17β-estradiol is the predominant estrogen in premenopausal women. Estriol has a weaker estrogenic activity, it is the predominant estrogen during pregnancy, but its role outside pregnancy has received little attention thus far [45]. The interpretation of our results is somewhat complicated by the fact that estriol binds to estrogen receptors as a partial antagonist in the presence of 17β-estradiol [46], but it appears that 17β-

estradiol and estriol can exert both stone promoting and stone inhibitory effects – stone promoting effects through a higher urinary calcium excretion and stone inhibitory effects through a higher urinary citrate excretion. Some of these effects seem to be influenceable by dietary habits and may therefore vary in the short to medium term. 17β-estradiol and estriol are synthesized from testosterone and androstenedione, respectively, by aromatase activity, which is reflected by the ratio testosterone/17β-estradiol (with a higher ratio corresponding to a lower enzyme activity). A significant interaction term with this ratio was found with age for the association with urinary oxalate, indicating an inverse association between oxalate excretion and aromatase activity in the elderly. The ratio testosterone/17β-estradiol also showed a significant interaction with NGIA for the association with urinary citrate, indicating an inverse association between citrate excretion and aromatase activity at a high NGIA. The significance of both findings remains unclear. Our results on estrogens are partially supported by previous studies in postmenopausal calcium oxalate stone formers showing a higher 24-hour urinary excretion of citrate and calcium, but not for oxalate, under ERT in one study [21], but a lower 24-hour urinary calcium excretion and no difference for the excretion of oxalate and citrate under ERT in another study [22]. However, it should be noted that our study population differs substantially from these studies, as we included 80% men, and only 39% of women were postmenopausal. In addition, differences in results may be explained by ERT related effects on bone or intestine, and even ERT related effects on the kidney may differ compared to the effects from endogenously derived estrogens at physiological levels. Results from animal studies and from *in vitro* data on the influence of estrogens on renal calcium handling and on potential underlying mechanism are conflicting [34].

Our analysis was conducted with urinary steroid hormone profiles on a random outpatient diet and urinary steroid profile data from kidney stone formers on different dietary regimes were not available. In addition to demographic characteristics and kidney function, we therefore

added three dietary factors to regression models in order to control for residual confounding due to individual dietary patterns among study participants. We have chosen NGIA as one dietary factor, for which a large influence on 24-hour urinary citrate excretion in stone-forming and healthy individuals is presumed [47]. In the present study, this influence expresses in a strong, positive and independent association between NGIA as explanatory co-variable and citrate as outcome variable, thereby corroborating previous findings [47]. We selected daily urinary sulfate and sodium excretion as two other potentially confounding dietary factors both known for their positive associations with daily urinary calcium excretion [48] and our results support the independency of both associations.

The cross-sectional design is a limitation of this study and does not allow interfering with causal relationships. Another limitation is that no data about the phase of menstrual cycle are available. 24-hour urines were collected outside the menstrual phase, and steroid hormone profiles from premenopausal women therefore derive from the follicular or luteal phase of the menstrual cycle. This may have an impact especially on urinary estrogens rather than on androgens or glucocorticoids as recently discussed by our group [32]. The phase of the menstrual cycle should be taken into account in future studies using urinary steroid hormone profiling.

In summary, our findings suggest that androgens and estrogens influence urinary calcium and citrate excretion. In addition, we identified DHEA as a novel factor associated with urinary oxalate excretion in humans.

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflicts of interest regarding this study. Results presented in this article have not been published previously in whole or part, except in abstract format. DGF has served as a consultant for Otsuka Pharmaceuticals and received unrestricted research funding from Novartis Otsuka Pharmaceuticals. DGF and NAD were supported by an unrestricted research grant from Abbvie.

AUTHORS' CONTRIBUTIONS

DGF, GAM and NAD conceived and planned the study. BV provided project resources. DGF and NAD recruited patients into the study. DGF, GAM, LS, CM, DL, and NAD conducted data curation. NAD performed statistical analyses. NAD, GAM, and DGF wrote the manuscript with input from all authors. All authors approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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Tables

Table 1. Characteristics of the study population. Characteristics are indicated for the whole study population and according to sex. Categorical variables are reported as number (N) and percentage. Continuous variables are reported as median; 25th-75th percentile and the number (N) indicates the number of available measurements. The N for urine solutes is reported after exclusion of incomplete urine collections and the N for sex steroid hormone metabolites is reported after the additional exclusion of extreme outliers as described in the methods section.

Variable	N	Men	N	Women	N	Total
Sex	402	80%	100	20%	502	100%
Age, years	402	47;37-56	100	42;34-54	502	46;36-56
Postmenopausal status (yes)	-	-	39	39%	-	-
BMI, kg/m^2	402	26;24-29	100	26;22-30	502	26;23-29
Diabetes (yes)	34	8%	10	10%	44	9%
Hypertension (yes)	207	51%	42	42%	249	50%
eGFR, mL/min per 1.73 m^2 BSA	399	97;83-109	98	102;86-113	497	97;83-110
24-hour urine volume, mL	402	2013;1491-2540	100	2108;1220-2613	502	2018;1464-2564
Creatinine, µmol/24h	376	15241;13202-17106	97	9535;7777-11280	473	14338;11657-16543
Urine sex hormone metabolites, nmol/24h						
Dehydroepiandrosterone (DHEA)	336	504;167-1783	92	226;106-794	428	460;152-1554
16α-OH-DHEA	369	975;470-2009	94	586;232-1246	463	861;391-1865
Androstenediol	352	451;241-892	94	270;130-468	446	393;203-821
Androstenetriol	369	1260;776-1928	95	894;458-1386	464	1167;710-1831
Testosterone	362	202;133-285	93	46;22-83	455	167;89-264
5α-DH-testosterone	365	96;62-134	93	43;27-77	458	84;52-127
Androstanediol	369	298;220-414	95	102;50-157	464	270;161-381
Androsterone	374	7155;4813-10309	95	3490;2010-5845	469	6270;4091-9734
11β-OH-androsterone	374	3132;2268-4411	97	2136;1279-2962	471	2951;2082-4131
Etiocholanolone	373	4763;3306-7030	96	3521;1784-5120	469	4580;2936-6821
17β-estradiol	369	8.6;6-12	89	8.2;4.5-13	458	8.6;5.8-12
Estriol	373	21;14-30	89	19;8.6-32	462	21;13-30
Kidney stone composition available	330	82%	76	76%	406	81%
Calcium oxalate (10-100%)	311	94%	63	83%	374	92%
Calcium phosphate (5-100%)	105	32%	28	37%	133	33%
Uric acid (10-100%)	21	6%	7	9%	28	7%
Cystine (20-100%)	7	2%	6	8%	13	3%
Urine solute excretion						
Sodium, mmol/24h	376	199;152-245	97	140;113-190	473	186;140-238
Calcium, mmol/24h	375	6.5;4.4-8.9	96	4.1;2.5-6	471	5.9;4-8.4
Oxalate, µmol/24h	370	433;307-592	96	285;210-405	466	403;278-576
Citrate, mmol/24h	370	2.8;1.8-3.8	96	2.1;1.3-3.2	466	2.6;1.7-3.7
Sulfate, mmol/24h	368	23;19-29	96	15;11-20	464	22;17-27
NGIA, mmol/24h	346	34;15-51	82	33;17-52	428	34;15-51
Calcium / Vitamin D supplement intake	9	2%	8	8%	17	3%
Thiazide intake	29	7%	9	9%	38	8%
Alkali intake	13	3%	8	8%	21	4%

Table 2. Association of urinary sex steroids and steroidogenic metabolite ratios with 24-hour urinary calcium excretion as outcome variable.

	Unadjusted Model			Multivariable Model							
	Main explanatory variable			Main explanatory variable			Explanatory co-variable			Interaction main explanatory variable with co-variable	
Explanatory variable	**N**	**β; 95% CI**	***p*-value**	**N**	**β; 95% CI**	***p*-value**		**β; 95% CI**	***p*-value**	**β; 95% CI**	***p*-value**
DHEA	427	0.20;0.11-0.3	2.5E-05	382	0.067;-0.024-0.16	0.15	NGIA	0.031;-0.055-0.12	0.48	-0.12;-0.20 to -0.035	0.0052
							Sulfate	0.29;0.20-0.39	1.7E-09	0.15;0.034-0.28	0.012
16α-OH-DHEA	461	0.18;0.089-0.27	1.2E-04	413	0.046;-0.051-0.14	0.35	eGFR	0.20;0.088-0.31	4.2E-04	-0.15;-0.24 to -0.056	0.0018
Androstenediol	445	0.22;0.13-0.31	2.0E-06	398	0.021;-0.072-0.11	0.66					
Androstenetriol	462	0.20;0.11-0.29	1.7E-05	413	0.070;-0.015-0.16	0.10					
Testosterone	453	0.33;0.24-0.42	1.7E-12	405	0.12;0.015-0.22	0.024					
5α-DH-testosterone	456	0.25;0.16-0.34	7.5E-08	410	0.061;-0.030-0.15	0.19	NGIA	0.031;-0.054-0.12	0.47	-0.10;-0.19 to -0.019	0.016
5α-Androstanediol	462	0.35;0.26-0.43	1.2E-13	415	0.10;0.0019-0.20	0.046					
Androsterone	467	0.32;0.23-0.40	4.6E-12	419	0.063;-0.042-0.17	0.24	Sulfate	0.35;0.25-0.45	3.4E-11	-0.19;-0.29 to -0.099	7.0E-05
11β-OH-androsterone	469	0.26;0.17-0.34	1.7E-08	420	0.13;0.042-0.22	0.0040	Sulfate	0.27;0.18-0.35	6.2E-09	-0.14;-0.24 to -0.035	0.0090
Etiocholanolone	467	0.21;0.12-0.30	5.2E-06	419	0.036;-0.056-0.13	0.44	NGIA	0.013;-0.071-0.097	0.76	-0.12;-0.20 to -0.043	0.0024
17β-estradiol	456	0.16;0.076-0.25	3.0E-04	408	0.13;0.043-0.21	0.0029					
Estriol	460	0.062;-0.030-0.15	0.19	413	0.047;-0.036-0.13	0.27	Sulfate	0.32;0.21-0.44	5.5E-08	-0.094;-0.18 to -0.0085	0.031
3β-HSD[1]	443	0.096;0.0020-0.19	0.045	395	-0.016;-0.11-0.08	0.74	Sulfate	0.34;0.23-0.45	2.3E-09	0.18;0.048-0.31	0.0077
CYP17A1 and 17,20-lyase[2]	440	-0.073;-0.16-0.018	0.12	395	0.033;-0.053-0.12	0.46					
Δ4- and Δ5-CYP17A1[3]	453	-0.063;-0.16-0.030	0.18	404	-0.069;-0.16-0.019	0.12					
Δ4-CYP17A1[4]	427	-0.11;-0.21 to -0.021	0.016	386	-0.052;-0.14-0.037	0.25					
Δ5-17,20-lyase[5]	398	0.0020;-0.095-0.099	0.97	355	-0.021;-0.11-0.064	0.63					
Δ4-17,20-lyase[6]	456	0.080;-0.012-0.17	0.089	408	-0.035;-0.12-0.053	0.43					
P450 oxidoreductase[7]	447	0.024;-0.071-0.12	0.62	399	-0.073;-0.16-0.018	0.12					
5α-reductase[8]	460	-0.14;-0.23 to -0.052	0.0021	412	-0.0040;-0.088-0.080	0.93	Sulfate	0.32;0.22-0.42	6.6E-10	0.14;0.05-0.22	0.0021
5α-reductase[9]	454	-0.13;-0.22 to -0.042	0.0044	405	-0.011;-0.096-0.075	0.81	Sulfate	0.32;0.22-0.43	2.1E-09	0.13;0.037-0.22	0.0060
5α-reductase[10]	456	-0.12;-0.21 to -0.028	0.010	408	-0.048;-0.13-0.035	0.25	Sulfate	0.31;0.20-0.41	1.0E-08	0.11;0.015-0.20	0.023
Aromatase[11]	427	0.11;0.019-0.21	0.019	383	-0.070;-0.17-0.029	0.17					

The number of participants, unadjusted and adjusted beta coefficients (β), their 95% confidence intervals (95% CI) and the corresponding *p*-values are indicated for each model. Steroidogenic enzymes are reflected by metabolite ratios and a higher ratio corresponds to a lower enzyme activity. All continuous variables in the models (explanatory variables and outcome variables) were scaled to standard deviation (SD). Therefore, every one SD increase in the explanatory variable results in an average increase of β SDs in each outcome. Multivariable models are adjusted for the co-variables sex (women versus men), age, BMI, eGFR, NGIA, sulfate and sodium, and, if significant, also for the interaction between co-variables with the main explanatory variable.
Footnotes: [1]Pregnenetriol/(THE+THF+5αTHF), [2](THA+THB+5αTHB)/(Androsterone+Etiocholanolone), [3](THA+THB+5αTHB)/(THE+THF+5αTHF), [4]Pregnanediol/(17αOHpregnanolone+Pregnanetriol), [5]Pregnenetriol/(DHEA+16αOHDHEA+Androstenediol+Androstenetriol), [6](17αOHpregnanolone+Pregnanetriol)/11βOHandrosterone, [7](17αOHpregnanolone+Pregnanetriol)/(THE+THF+5αTHF), [8]Androsterone/Etiocholanolone, [9]THF/5αTHF, [10]THB/5αTHB,[11]Testosterone/17βestradiol
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; NGIA, net gastrointestinal alkali absorption; Sulfate, 24-hour urinary sulfate excretion; Sodium, 24-hour urinary sodium excretion; DHEA, dehydroepiandrosterone; 3β-HSD, 3β-hydroxysteroid dehydrogenase, CYP17A1, 17α-hydroxylase; Δ4 and Δ5, Delta 4 and Delta 5 steroidogenic pathways; THE, Tetrahydrocortisone; THF, Tetrahydrocortisol; THA, Tetrahydro-11-dehydro-corticosterone; THB, Tetrahydrocorticosterone

Table 3. Association of urinary sex steroids and steroidogenic metabolite ratios with 24-hour urinary oxalate excretion as outcome variable.

	Unadjusted Model			Multivariable Model						
	Main explanatory variable			Main explanatory variable			Explanatory co-variable			Interaction main explanatory variable with co-variable	
Explanatory variable	**N**	**β; 95% CI**	***p*-value**	**N**	**β; 95% CI**	***p*-value**		**β; 95% CI**	***p*-value**	**β; 95% CI**	***p*-value**
DHEA	421	-0.018;-0.12-0.082	0.73	382	-0.11;-0.21 to -0.0050	0.040					
16α-OH-DHEA	456	-0.024;-0.12-0.069	0.61	413	-0.05;-0.15-0.053	0.34					
Androstenediol	440	0.041;-0.054-0.14	0.40	398	-0.028;-0.13-0.077	0.60					
Androstenetriol	457	0.068;-0.024-0.16	0.15	413	0.038;-0.055-0.13	0.42					
Testosterone	448	0.16;0.063-0.25	0.0011	405	0.077;-0.036-0.19	0.18					
5α-DH-testosterone	451	0.11;0.013-0.20	0.025	410	0.012;-0.090-0.11	0.81					
5α-Androstanediol	457	0.21;0.12-0.30	8.7E-06	415	0.11;-0.0049-0.22	0.061					
Androsterone	462	0.15;0.056-0.24	0.0017	419	0.071;-0.045-0.19	0.23					
11β-OH-androsterone	464	0.19;0.097-0.28	5.1E-05	420	0.078;-0.018-0.17	0.11					
Etiocholanolone	462	0.13;0.034-0.22	0.0079	419	0.051;-0.051-0.15	0.32					
17β-estradiol	451	0.014;-0.078-0.11	0.77	408	-0.033;-0.13-0.06	0.49					
Estriol	455	0.025;-0.068-0.12	0.60	413	-0.013;-0.11-0.078	0.77					
3β-HSD[1]	437	-0.063;-0.16-0.031	0.19	395	-0.058;-0.16-0.047	0.28					
CYP17A1 and 17,20-lyase[2]	435	-0.056;-0.15-0.040	0.25	395	-0.028;-0.12-0.069	0.57					
Δ4- and Δ5-CYP17A1[3]	448	-0.080;-0.17-0.012	0.088	404	0.028;-0.070-0.13	0.58	Sulfate	0.11;0.015-0.20	0.024	0.14;0.0019-0.27	0.047
Δ4-CYP17A1[4]	422	-0.16;-0.26 to -0.066	0.0010	386	-0.054;-0.15-0.045	0.28					
Δ5-17,20-lyase[5]	393	-0.0070;-0.11-0.096	0.89	355	-0.0093;-0.11-0.09	0.85					
Δ4-17,20-lyase[6]	451	-0.0090;-0.1-0.085	0.85	408	-0.013;-0.11-0.084	0.79					
P450 oxidoreductase[7]	442	0.014;-0.080-0.11	0.77	399	0.067;-0.032-0.17	0.19					
5α-reductase[8]	455	-0.028;-0.12-0.063	0.54	412	0.018;-0.074-0.11	0.70					
5α-reductase[9]	449	-0.063;-0.15-0.026	0.16	405	-0.019;-0.11-0.072	0.69					
5α-reductase[10]	451	-0.043;-0.13-0.048	0.35	408	-0.0005;-0.091-0.090	0.99					
Aromatase[11]	422	0.16;0.065-0.26	0.0012	383	0.18;0.068-0.29	0.0018	Age	0.11;-0.027-0.24	0.12	0.15;0.052-0.25	0.0027

Explanations, footnotes and abbreviations: Please refer to Table 2.

Table 4. Association of urinary sex steroids and steroidogenic metabolite ratios with 24-hour urinary citrate excretion as outcome variable.

	Unadjusted Model			Multivariable Model						
	Main explanatory variable			Main explanatory variable			Explanatory co-variable			Interaction main explanatory variable with co-variable	
Explanatory variable	**N**	**β; 95% CI**	***p*-value**	**N**	**β; 95% CI**	***p*-value**		**β; 95% CI**	***p*-value**	**β; 95% CI**	***p*-value**
DHEA	421	-0.048;-0.15-0.050	0.34	382	-0.063;-0.16-0.037	0.22					
16α-OH-DHEA	456	0.042;-0.052-0.14	0.38	413	0.10;0.004-0.20	0.042					
Androstenediol	440	-0.034;-0.13-0.059	0.47	398	-0.041;-0.14-0.060	0.43					
Androstenetriol	457	0.035;-0.057-0.13	0.46	413	0.088;-0.0033-0.18	0.059					
Testosterone	448	0.11;0.019-0.21	0.018	405	0.13;0.023-0.24	0.018					
5α-DH-testosterone	451	0.098;0.0050-0.19	0.039	410	0.052;-0.046-0.15	0.30					
5α-Androstanediol	457	0.069;-0.024-0.16	0.14	415	-0.0088;-0.12-0.10	0.87					
Androsterone	462	0.086;-0.0060-0.18	0.068	419	0.15;0.040-0.27	0.0081	Sulfate	0.18;0.069-0.29	0.0015	-0.15;-0.25 to -0.042	0.0059
11β-OH-androsterone	464	0.13;0.036-0.22	0.0063	420	0.063;-0.031-0.16	0.19					
Etiocholanolone	462	0.045;-0.049-0.14	0.35	419	0.033;-0.067-0.13	0.52					
17β-estradiol	451	0.17;0.079-0.26	2.4E-04	408	0.065;-0.026-0.16	0.16	Sodium	0.021;-0.081-0.12	0.69	-0.11;-0.20 to -0.018	0.020
Estriol	455	0.26;0.17-0.35	2.4E-08	413	0.22;0.12-0.33	4.0E-05	Sex(women)	-0.34;-0.57 to -0.11	0.0044	-0.21;-0.41 to -0.013	0.037
							Sodium	0.043;-0.057-0.14	0.40	-0.14;-0.23 to -0.044	0.0040
3β-HSD[1]	437	-0.066;-0.16-0.030	0.18	395	0.012;-0.093-0.12	0.82					
CYP17A1 and 17,20-lyase[2]	435	0.010;-0.084-0.11	0.83	395	-0.014;-0.11-0.080	0.78	NGIA	0.29;0.19-0.38	4.1E-09	0.12;0.021-0.21	0.017
Δ4- and Δ5-CYP17A1[3]	448	-0.053;-0.15-0.042	0.27	404	-0.054;-0.16-0.052	0.32	Sex(women)	-0.36;-0.59 to -0.12	0.0033	0.31;0.11-0.52	0.0031
Δ4-CYP17A1[4]	422	-0.013;-0.11-0.083	0.79	386	0.077;-0.018-0.17	0.11	NGIA	0.28;0.18-0.37	1.9E-08	-0.11;-0.21 to -0.011	0.030
Δ5-17,20-lyase[5]	393	-0.013;-0.11-0.085	0.79	355	-0.020;-0.11-0.074	0.68					
Δ4-17,20-lyase[6]	451	0.049;-0.045-0.14	0.31	408	0.042;-0.053-0.14	0.38					
P450 oxidoreductase[7]	442	0.013;-0.082-0.11	0.78	399	0.034;-0.064-0.13	0.50					
5α-reductase[8]	455	-0.032;-0.12-0.059	0.49	412	-0.021;-0.11-0.070	0.65					
5α-reductase[9]	449	-0.11;-0.20 to -0.019	0.018	405	-0.066;-0.16-0.027	0.16					
5α-reductase[10]	451	-0.044;-0.14-0.048	0.35	408	-0.0098;-0.099-0.08	0.83					
Aromatase[11]	422	-0.038;-0.13-0.059	0.45	383	0.025;-0.083-0.13	0.65	NGIA	0.29;0.19-0.39	8.2E-09	0.091;0.0092-0.17	0.029

Explanations, footnotes and abbreviations: Please refer to Table 2.

FIGURES

Figure 1.

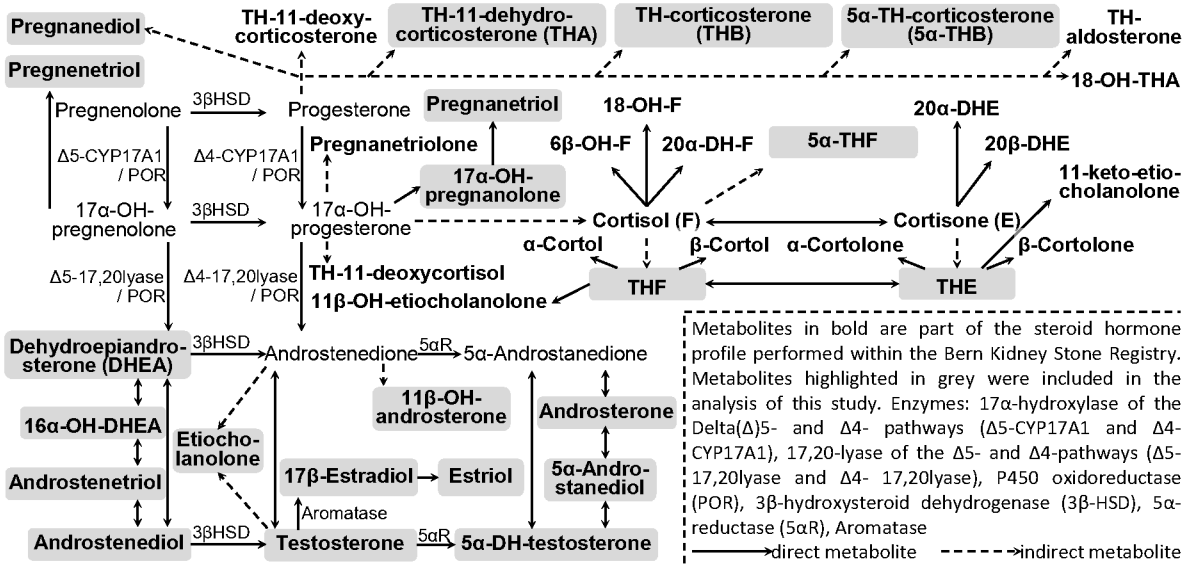

Figure 1. Important metabolic pathways of steroid hormone biosynthesis in humans. The depicted steroidogenic pathways start with pregnelonone in the upper left corner and continue towards sex steroid hormone biosynthesis in the lower part of the figure and towards biosynthesis of corticosterones, mineralocorticoids and glucocorticoids in the left part of the figure. DHEA and its sulfonated form are the most abundant circulating steroid hormones and androgen precursors, whereas androsterone together with etiocholanolone are the main urinary androgen metabolites standing at the end of the metabolic pathway [49-51]. Most of testosterone and 5α-DH-testosterone is thought to be synthetized through the so called Δ5-pathway, which runs from 17α-hydroxy-pregnenolone via DHEA to androstenedione and involves 17α-hydroxylase, 17,20-lyase, and P450 oxidoreductase enzymes. The Δ4-pathway from progesterone via 17-OH-progesterone to androstenedione likely contributes little to the overall androgen production in humans.

Figure 2

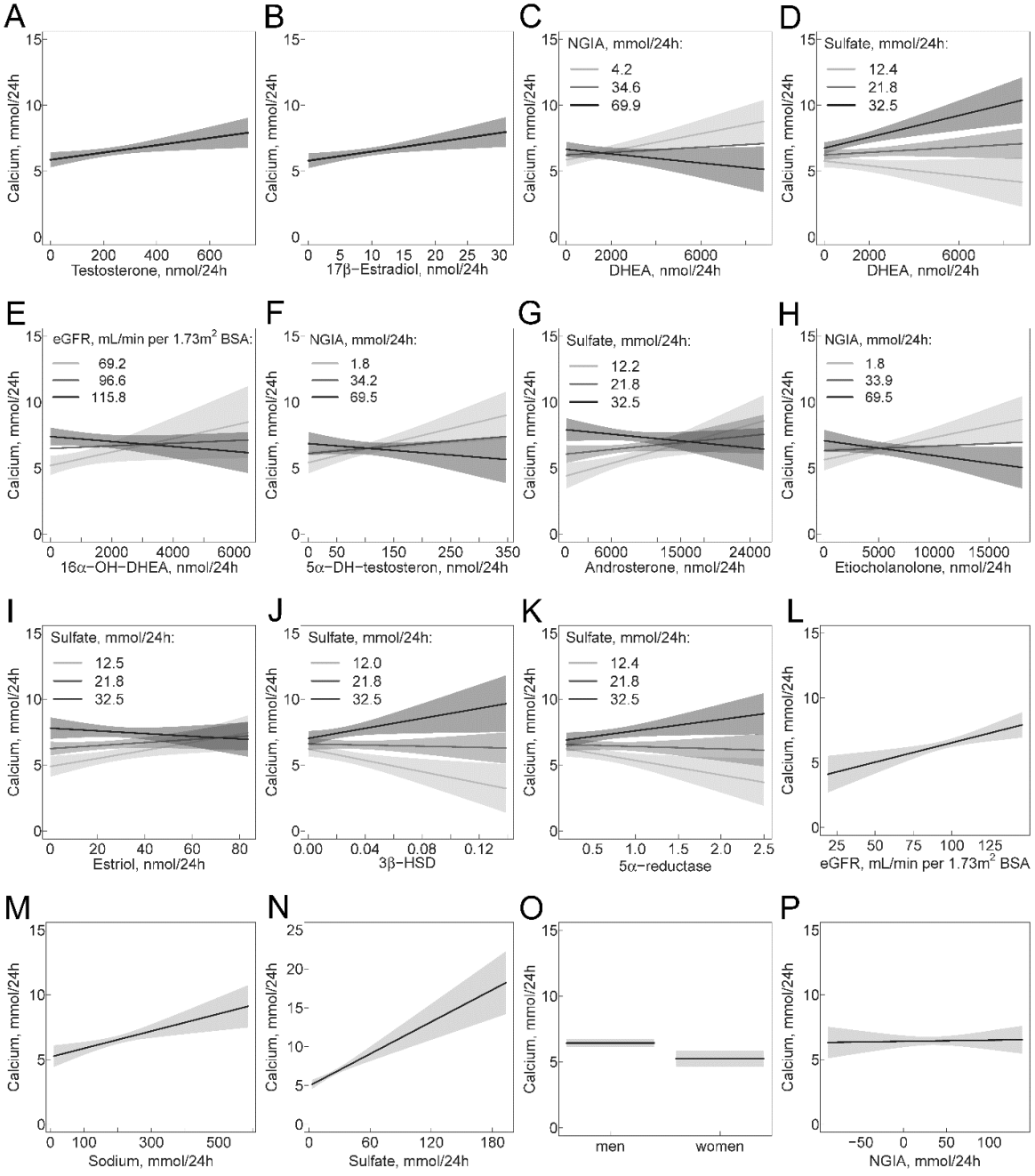

Figure 2. Association between urinary sex steroids, steroidogenic metabolite ratios and other predictors with 24-hour urinary calcium excretion. Adjusted regression models from Table 2 are visualized showing the relationship between the explanatory variable of interest on the x-axis and urinary calcium excretion as outcome variable on the y-axis while holding the effect of all other co-variables constant. All models are adjusted for the co-variables sex, age, BMI, eGFR, NGIA, urinary sulfate and urinary sodium. Solid lines

represent regression lines and shaped areas the surrounding 95% CI band. In **Figure 2C-2L**, represented models contain interaction terms with NGIA, sulfate, or eGFR and the visualized associations depend on the level of these effect modifiers. Effect modifiers were splitted into tertiles with mean values indicated at the top of each diagram. In **Figure 2C, Figure 2E, Figure 2F, and Figure 2H** the 95% CI band of the center tertile of the effect modifier was omitted for better clarity.

Figure 3

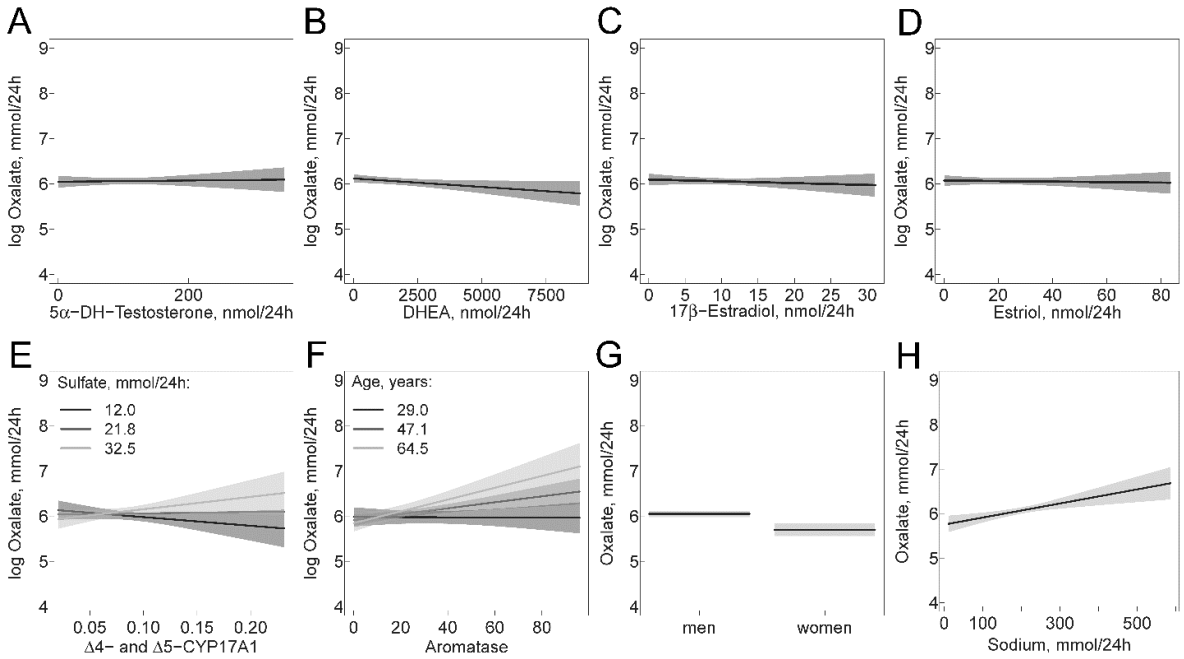

Figure 3. Association between urinary sex steroids, steroidogenic metabolite ratios and other predictors with 24-hour urinary oxalate excretion. Adjusted regression models from Table 3 are visualized showing the relationship between the explanatory variable of interest on the x-axis and urinary oxalate excretion as outcome variable on the y-axis while holding the effect of all other co-variables constant. All models are adjusted for the co-variables sex, age, BMI, eGFR, NGIA, urinary sulfate and urinary sodium. Solid lines represent regression lines and shaped areas the surrounding 95% CI band. In **Figure 2E** and **Figure 3F**, underlying models contain interaction terms with sulfate and age and the visualized associations depend on the level of these effect modifiers. Effect modifiers were splitted into tertiles with mean values indicated at the top of each diagram. In **2E** the 95% CI band of the center sulfate-tertile was omitted for better clarity.

Figure 4

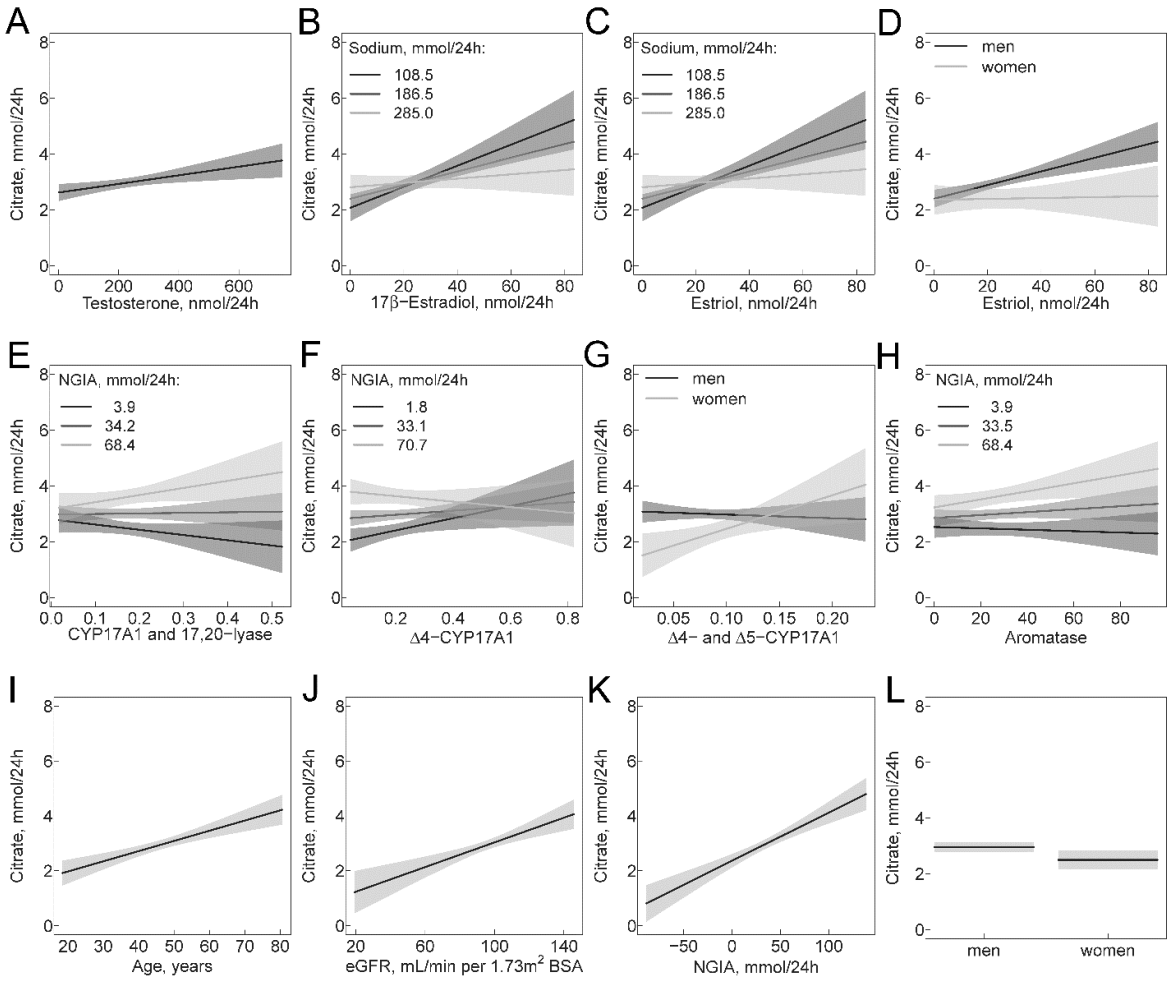

Figure 4. Association between urinary sex steroids, steroidogenic metabolite ratios and other predictors with 24-hour urinary citrate excretion. Adjusted regression models from Table 4 are visualized showing the relationship between the explanatory variable of interest on the x-axis and urinary citrate excretion as outcome variable on the y-axis while holding the effect of all other co-variables constant. All models are adjusted for the co-variables sex, age, BMI, eGFR, NGIA, urinary sulfate and urinary sodium. Solid lines represent regression lines and shaped areas the surrounding 95% CI band. In **Figure 2B-2H**, models contain interaction terms with sulfate, sodium, sex, and NGIA and the visualized associations depend on the level of these effect modifiers. Continuous effect modifiers were splitted into tertiles with mean values indicated at the top of each diagram. In **Figure 2B** and **Figure 2C** the 95% CI bands of the center sodium-tertiles were omitted for better clarity.