

This is the overview page

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

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3 1 **Association of urinary sex steroid hormones with urinary calcium, oxalate and citrate**
4 2 **excretion in kidney stone formers**
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33 19 **Running Head:**

34 20 Sex hormones and urinary lithogenic risk factors
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3 22 **ABSTRACT**

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5 23 **Background.** Sex-specific differences in nephrolithiasis with respect to both distribution of
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7 24 prevalence and stone composition are widely described and may be influenced by sex
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9 25 hormones.

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11 26 **Methods.** We conducted a cross-sectional analysis of the relationship between 24-hour urinary
12
13 27 sex hormone metabolites measured by gas chromatography–mass spectrometry with urinary
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15 28 calcium, oxalate and citrate excretion in a cohort of 628 kidney stone formers from a tertiary
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17 29 care hospital in Switzerland, taking demographic characteristics, kidney function and dietary
18
19 30 factors into account.

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21
22 31 **Results.** We observed a positive association of urinary calcium with urinary testosterone and
23
24 32 17β -estradiol. Positive associations of urinary calcium with dehydroepiandrosterone, 5α -DH-
25
26 33 testosterone, etiocholanolone, androsterone, and estriol were modified by net gastrointestinal
27
28 34 alkali absorption or urinary sulfate excretion. As the only sex hormone, dehydroepiandrosterone
29
30 35 was inversely associated with urinary oxalate excretion in adjusted analyses. Urinary citrate
31
32 36 correlated positively with urinary testosterone. Associations of urinary citrate with urinary
33
34 37 androsterone, 17β -estradiol and estriol were modified by urinary sulfate or sodium, or by sex.

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37 38 **Conclusions.** Urinary androgens and estrogens are significantly associated with urinary
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39 39 calcium and citrate excretion, and associations are in part modified by diet. Our data
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41 40 furthermore reveal dehydroepiandrosterone as a novel factor associated with urinary oxalate
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43 41 excretion in humans.

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3 **43 What is already known about this subject**

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5 44 Little is known about the influence of sex hormones on lithogenic risk factors in humans and
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7 45 available data are conflicting. This may at least partially be due to limitations of analytical
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9 46 methods used for sex steroid hormone analyses in the past including immunoassays that are
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11 47 known for lower specificity compared to chromatography-mass spectrometry techniques. Also,
12
13 48 blood levels of steroid hormones depend on the timing of blood sampling, thus, they likely not
14
15 49 comprehensively represent daily production of sex steroid hormones in kidney stone formers.

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18 **51 What this study adds**

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20 52 Here we quantified twelve urinary sex steroid hormones in 24-hour urines using gas
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22 53 chromatography–mass spectrometry in a well-characterized large cohort of 628 kidney stone
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24 54 formers and assessed the association with urinary calcium, oxalate and citrate excretion. Our
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26 55 data demonstrate that androgens and estrogens are predictors of urinary calcium and citrate
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28 56 excretion in humans and may exert both stone promoting and stone inhibitory effects, and some
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30 57 of these effects seem to be influenceable by dietary habits. Our data furthermore reveal
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32 58 dehydroepiandrosterone as a novel factor associated with urinary oxalate excretion in humans.

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35 **60 What impact this may have on practice or policy**

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37 61 These results hint at an interplay between sex hormones and dietary factors on urine
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39 62 lithogenicity and may encourage further research efforts in this direction. Determining urinary
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41 63 sex steroid hormones together with dietary markers may improve future risk assessment in
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43 64 kidney stone formers. Additionally, it may encourage to place further emphasis on dietary
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45 65 advices and guidance as modifiable factors in clinical practice.

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47 66
48 **67 Keywords:** sex steroid hormones, nephrolithiasis, lithogenic risk factors, dietary factors
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69 INTRODUCTION

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

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

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

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3 93 Thus, previous studies yielded conflicting results with respect to the association of sex
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5 94 hormones with stone formation rate or urinary lithogenic risk factors. This may be at least
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7 95 partially due to methodological reasons. Different analytical methods were used for sex steroid
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9 96 hormone analyses in the past, among them immunoassays, that are known for lower specificity
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11 97 compared to chromatography-mass spectrometry techniques [23]. In addition, blood levels of
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13 98 steroid hormones depend on the timing of blood sampling, thus, they likely not
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15 99 comprehensively represent the daily production of sex steroid hormones.

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18 100 Specific objectives of this study were to assess in 24-hour urine samples of kidney stone formers
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20 101 the relationship of urinary sex hormones and their metabolites, measured by gas
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22 102 chromatography-mass spectrometry (GC-MS), with urinary calcium, oxalate and citrate
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24 103 excretion.
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28 105 **MATERIALS AND METHODS**

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31 106 Bern Kidney Stone Registry participants were recruited at the Division of Nephrology and
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33 107 Hypertension at the Bern University Hospital, Bern, Switzerland and underwent a
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35 108 comprehensive metabolic workup including 24-hour urinary steroid hormone profiling [24, 25].
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37 109 The Bern Kidney Stone Registry adheres to the Declaration of Helsinki and was approved by
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39 110 the Ethical Committee of the Kanton Bern (# 95/06). All participants provided written informed
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41 111 consent. Kidney stone patients at least 18 years of age, suffering from at least one stone episode
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43 112 and with an available urinary steroid hormone profile, in total 628 patients, were eligible for
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45 113 analysis. Participants with characteristics that may strongly influence calciuria, oxaluria and /
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47 114 or citraturia were excluded from analysis: history of primary hyperparathyroidism (n=16), a
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49 115 total plasma calcium >2.5mmol/L with parathyroid hormone (PTH) >65 pg/mL (n=20),
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51 116 sarcoidosis (n=3), pregnancy or lactation during the study visit (n=2), anorexia nervosa or
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54 117 bulimia (n=3), active malignant diseases (n=15), solid organ transplantation (n=2), the intake
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3 118 of phosphate supplementation (n=1), bisphosphonates (n=11), cinacalcet (n=1), denusomab
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5 119 (n=1), teriparatide (n=1), loop diuretics (n=10), potassium-sparing diuretics (n=9), or
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7 120 carboanhydrase inhibitors (n=1). Additionally, participants with conditions interacting with
8
9 121 steroid hormone metabolism were excluded from analysis: diagnosis of polycystic ovary
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11 122 syndrome (n=1), history of unilateral or bilateral oophorectomy (n=1) or orchiectomy (n=1),
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13 123 use of the following fix or on-demand medication: topic or systemic glucocorticoids (n=24),
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15 124 hormones used for menopause treatment, to prevent conception or to suppress menstrual
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17 125 bleeding (n=32), use of anabolic steroids (n=1), anti-androgen or anti-estrogen therapy (n=7),
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19 126 antiepileptic agents (n=3). Finally, 126 patients were excluded from analysis.

22 127 **Laboratory measurements and definitions**

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24 128 The urinary steroid profile consists of 40 steroid hormones and metabolites, including 12 sex
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26 129 steroid hormones and metabolites, and was quantified in a 24-hour urine collection on a random
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28 130 outpatient diet by an *in-house* adapted GC-MS method in the steroid laboratory of the
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30 131 Department of Nephrology and Hypertension at the Bern University Hospital, Switzerland [26,
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32 132 27]. Established steroid hormone metabolite ratios reflecting steroidogenic enzyme activities
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34 133 towards sex steroid hormone biosynthesis (**Figure 1**) were calculated from steroid hormone
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36 134 compounds [28, 29]. All other urinary parameters were measured centrally by standard clinical
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38 135 laboratory methods from two 24-hour urine collections on two consecutive days. Urine
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40 136 collections with a creatinine excretion value outside a predefined 2.5th-97.5th confidence
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42 137 interval based on urinary creatinine excretion values from the adult Swiss population were
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44 138 regarded as under- or overcollected and were therefore excluded from the analysis [30]. Mean
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46 139 24-hour excretion values from two urine collections, if available, were used for statistical
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48 140 analysis. The 24-hour urinary sodium excretion was used to assess salt intake. Net
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50 141 gastrointestinal alkali absorption (NGIA) was calculated from 24-hour urinary electrolytes [31].
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52 142 The 24-hour urinary sulfate excretion was used as a marker of dietary acid ash content from
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3 143 sulfur-containing amino acids. Fourier transform infrared spectroscopy was performed for
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5 144 kidney stone analysis.

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10 11 147 **Statistical analysis**

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13 148 Extreme outliers of each steroid hormone metabolite were defined as outside the range of the
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15 149 25th percentile minus 3×interquartile range to the 75th percentile plus 3×interquartile range and
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17 150 were also excluded from analysis. A descriptive statistic was created, which included the
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19 151 available data for each variable of interest for the whole study population and also dichotomized
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21 152 by sex. Sex-specific differences in urinary excretion of sex hormone metabolites, calcium,
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23 153 oxalate and citrate were assessed by Wilcoxon rank-sum test. Type and strength of relationship
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25 154 between two sex hormone metabolites was explored by visual inspection of bivariate
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27 155 scatterplots and by calculation of bivariate Spearman's and Pearson's correlation. The potential
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29 156 influence of sex steroid hormone metabolites as main predictor variables on the 24-hour
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31 157 excretion of the three lithogenic solutes calcium, oxalate and citrate as outcome variables was
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33 158 assessed by univariate and adjusted linear regression analyses. To fit linear model assumptions,
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35 159 the outcome variables calcium and citrate were square root-transformed and the outcome
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37 160 variable oxalate was log-normal transformed. All continuous predictor variables and all
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39 161 outcome variables were scaled for regression analyses. Adjusted analyses included the seven
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41 162 co-variables sex, age, body mass index (BMI), eGFR, NGIA, sulfate and sodium as potential
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43 163 confounders. To account for potential effect modifications, significant interaction terms
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45 164 between the main predictor variables and the co-variables were added to adjusted regression
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47 165 models by forward selection analyses. Adjusted beta coefficients and corresponding 95%
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49 166 confidence intervals were calculated and reported. A *P* value <0.05 was considered to be
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51 167 significant. Models were validated by graphical analysis for homogeneity of variance,
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3 168 normality of residuals, and highly influential observations. All final adjusted models were
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5 169 further tested for their robustness by restricting the adjusted analysis to a subset of the study
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7 170 population without the following additional potential confounders: intake of over-the-counter
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9 171 or prescribed calcium and/or vitamin D supplements (n=17), thiazide diuretics (n=38),
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11 172 potassium or magnesium citrate or sodium bicarbonate (n=21) and postmenopausal status (n=39
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13 173 out of n=100 women). Interaction terms that did not persist robustly in this sensitivity analysis
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15 174 were omitted from the final adjusted models. All statistical analyses were performed with R
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17 175 software, version 3.5.0 (R Foundation for Statistical Computing).
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22 177 **RESULTS**

23 178 **Characteristics of the study population**

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26 179 Characteristics of 502 kidney stone formers included in the final analysis are shown in **Table**

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29 180 **1.** Age of study participants ranged from 18 to 80 years, and the percentage of female stone
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31 181 formers was 20.3% (N=103), among them 38.8% (N=40) self-reported post-menopausal
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33 182 women. Urinary sex steroid metabolites exhibited a right-skewed distribution and median
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35 183 values of each sex group were within the range of reference intervals recently published for
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37 184 men and women [32]. The excreted amount of all ten androgen metabolites was substantially
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39 185 higher in men than in women across all age groups, whereas no sex specific differences were
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41 186 found for the excretion of the two estrogen metabolites 17 β -estradiol and estriol. Kidney stone
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43 187 analysis was available in 80.9% (N=406) of patients, and most of the stones contained calcium
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45 188 oxalate (92%). Higher 24-hour excretions in men than in women were found for calcium,
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47 189 oxalate and citrate. Correlation analyses between sex hormone metabolites revealed a strong
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49 190 positive linear relationship of dehydroepiandrosterone (DHEA) with its two direct metabolites
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51 191 androstenediol and 16 α -OH-DHEA, a moderate positive relationship of testosterone and 17 β -
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53 192 estradiol with their direct metabolites 5 α -DH-testosterone and estriol, respectively, and
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3 193 throughout weak positive relationships between androgen metabolites and estrogen metabolites
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5 194 (**Supplementary Table S1**).

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11 197 **Association analysis with calcium excretion as outcome variable**

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13 198 In unadjusted analyses, a positive association of urinary calcium excretion was found with
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15 199 urinary excretion of all ten androgen metabolites and with 17 β -estradiol, but not with estriol
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17 200 (**Table 2**). After multivariable adjustment (**Table 2 and Figures 2A-2P**), the association with
18
19 201 testosterone, 5 α -androstane-3 β -diol, 11 β -OH-androsterone, and 17 β -estradiol remained robust. For
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21 202 the association with urinary calcium excretion, significant interactions with the dietary markers
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23 203 NGIA or sulfate were found for the most abundant circulating androgen metabolite DHEA, for
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25 204 the most potent androgen 5 α -DH-testosterone, for the most abundant urinary androgen
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27 205 metabolites androsterone and etiocholanolone, for estriol, and for hormone metabolite ratios.
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29 206 The described interaction effects remained robust in sensitivity analyses (**Supplementary**
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31 207 **Table S2**).

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35 208 Significant positive associations were found with the co-variables eGFR (range of β
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37 209 coefficients: 0.21-0.25, range of p -values: 2.2×10^{-4} - 8.9×10^{-6}), with urinary excretion of sodium
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39 210 (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
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41 211 significantly lower urinary calcium excretion was found in women compared to men (β : -0.50
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43 212 to -0.33, p : 1.1×10^{-5} -0.0078).

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48 214 **Association analysis with urinary oxalate excretion as outcome variable**

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50 215 In unadjusted analyses, positive associations of urinary oxalate excretion were found with the
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52 216 urinary excretion of six androgen metabolites (**Table 3**), including testosterone. In
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54 217 multivariable analyses (**Table 3 and Figures 3A-3H**), associations with androgen metabolites

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3 218 including 5 α -DH-testosterone did not remain significant. The association with DHEA became
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5 219 significantly negative. No significant association was found with the estrogen metabolites 17 β -
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7 220 estradiol and estriol. For steroidogenic enzyme activities, significant interactions were found
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9 221 for the 17 α -hydroxylase activity of the combined Δ^4 - and Δ^5 -pathways with sulfate excretion
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11 222 and for the aromatase activity with age. Interaction effects were robust in sensitivity analyses
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13 223 **(Supplementary Table S3).**

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15 224 With regard to co-variables in regression models, a significant lower oxalate excretion was
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17 225 found in women compared to men (β -range: -0.60 to -0.47, p -range: 3.4×10^{-6} - 5.3×10^{-4}) and a
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19 226 significant positive association was found with urinary excretion of sodium (β -range: 0.18-0.23,
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21 227 p - range: 9.5×10^{-5} - 5.6×10^{-4}).
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26 229 **Association analysis with urinary citrate excretion as outcome variable**

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28 230 In unadjusted analyses, a positive association of urinary citrate excretion was found with the
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30 231 three androgen metabolites testosterone, 5 α -DH-testosterone, and 11 β -OH-androsterone and
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32 232 with both estrogen metabolites 17 β -estradiol and estriol (**Table 4**). In multivariable adjustment
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34 233 (**Table 4 and Figures 4A-4L**), the association with testosterone remained robust and the
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36 234 positive association with 16 α -OH-DHEA became significant. For the association with urinary
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38 235 citrate, significant interactions were found with the dietary markers sulfate for androsterone,
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40 236 with sodium for both estrogen metabolites, and with NGIA for hormone metabolite ratios.
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42 237 Interaction effects were robust in sensitivity analyses (**Supplementary Table S4**).
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46 238 The assessment of co-variables revealed a significant positive associations with age (β -range:
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48 239 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-
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50 240 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 -
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52 241 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
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3 242 significant lower citrate excretion was found in women compared to men (β -range: -0.27 to -
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5 243 0.36, p -range: 0.028-0.0044).
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12 13 247 **DISCUSSION**

14
15 248 To our knowledge, this is the first study within the area of kidney stone research that considers
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17 249 such a rich phenotype of steroid hormone metabolism in relation to the most important
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19 250 predictive solutes of calcium oxalate supersaturation as the driving force for calcium stone
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21 251 formation. Our results reveal an association of urinary androgens on urinary calcium excretion.
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23 252 This may be due to a direct effect of androgens on renal calcium transporters, as previously
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25 253 reported in mice [33]. Compared to sham-operated controls, orchidectomized mice displayed
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27 254 reduced urinary calcium excretion accompanied by upregulated mRNA and protein levels of
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29 255 the luminal transient receptor potential vanilloid-subtype 5 channel (TRPV5) and of the
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31 256 intracellular calcium-binding protein calbindin-D_{28K} in the distal convoluted tubule (DCT).
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33 257 Treatment of orchidectomized mice with testosterone normalized both urinary calcium
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35 258 excretion and the expression of TRPV5 and calbindin-D_{28K}. Serum levels of calcium,
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37 259 parathyroid hormone, 1,25-dihydroxyvitamin D₃, and estrogen did not differ between treatment
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39 260 groups, suggesting a direct effect of testosterone on renal calcium handling. This notion is
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41 261 further supported by *in vitro* experiments – treatment with 5 α -DH-testosterone led to an
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43 262 upregulation of TRPV5 in primary tubular cells [33]. Sex steroid hormone receptors are
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45 263 expressed in the kidney of women and men [34]. In addition, the enzymes 3 β -hydroxysteroid
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47 264 dehydrogenase, 17 α -hydroxylase/17,20-lyase, P450 oxidoreductase, 5 α -reductase, and
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49 265 aromatase, which are all essential for sex steroid hormone biosynthesis, are expressed in the
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51 266 kidney [35-37]. Therefore, the additional presence of an extragonadal sex steroid hormone
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3 267 metabolism in the human kidney appears to be very probable. Beside a direct action on the
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5 268 kidney, however, sex steroids may also influence renal calcium excretion indirectly e.g. by
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7 269 affecting calcium metabolism in gut or bone.

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9 270 Adjusted analyses in our study revealed positive associations between urinary calcium
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11 271 excretion with DHEA at a lower NGIA level and at a higher urinary sulfate level, indicating
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13 272 that the association is highly dependent on dietary alkali (high NGIA, low sulfate) and acid
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15 273 (low NGIA, high sulfate) intake. In addition, positive associations between urinary calcium
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17 274 with 5 α -DH-testosterone and etiocholanolone at a lower NGIA level and with androsterone at
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19 275 a lower sulfate level were found. Thus, apparent contrary results were obtained for the
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21 276 interaction effects between sulfate and DHEA and sulfate and androsterone.

22
23 277 Positive associations of urinary oxalate with androgen metabolites observed in unadjusted
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25 278 analysis did not remain significant after adjustment. Addition of the co-variable sex to
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27 279 univariable regression models caused the largest change in the association between androgen
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29 280 metabolites and oxalate excretion. Thus, sex was the key confounding variable in the models.
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31 281 In a group of 48 men from India with calcium nephrolithiasis, 24-hour urinary oxalate
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33 282 correlated weakly with free serum testosterone (Pearson's $r=0.297$, p -value=0.040) [38]. No
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35 283 adjusted analyses were performed in this study, however, likely due to the small number of
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37 284 study participants. Our results demonstrate that even strong associations between androgens
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39 285 and oxalate excretion in unadjusted analyses may not be robust to multivariable adjustment due
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41 286 to strong confounders.

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43 287 In contrast, DHEA and its main metabolite 16 α -OH-DHEA showed a negative association with
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45 288 oxalate, and for DHEA this association became significant in adjusted analyses. Together with
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47 289 its sulfonated form, DHEA is the most abundant circulating steroid hormone exhibiting weak
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49 290 androgenic activity, but its physiological role in brain and liver exceeds those of a simple sex
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51 291 steroid precursor and comprises numerous signaling pathways [39]. Endogenously produced
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3 292 oxalate derives from glyoxalate. Glyoxalate is formed in the liver from glycolate by glycolate
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5 293 oxidase (GO) and can be converted to glycolate by glyoxylate/hydroxypyruvate reductase
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7 294 (GRHPR) or to glycine by alanine-glyoxylate aminotransferase (AGXT) [40]. DHEA-treated
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9 295 castrated rats had higher hepatic enzyme activities of GRHPR and AGXT [41]. Hence, DHEA
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11 296 may directly modulate endogenous oxalate production in the liver.

12
13 297 We also observed a positive association of urinary citrate, a major inhibitor of calcium kidney
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15 298 stone formation, with testosterone and with androsterone at a low urinary sulfate excretion. This
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17 299 finding is in line with results from patients with polycystic ovary syndrome (PCOS) with high
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19 300 free serum testosterone which had higher 24-hour urinary citrate excretion compared to the
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21 301 subgroup with low free serum testosterone [42]. The authors of this study speculated that higher
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23 302 citraturia may have been induced by metabolic alkalosis due to increased mineralocorticoid
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25 303 activity with PCOS. To test this in our study, we added plasma bicarbonate as an additional co-
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27 304 variable to adjusted analysis of the association between urinary testosterone and citrate,
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29 305 however, the association remained essentially unchanged, indicating that plasma bicarbonate
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31 306 was not an additional confounding factor nor an effect modifier for the association.
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33 307 Physiologically, an increase of urinary citrate excretion makes absolute sense in a situation
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35 308 when urinary calcium excretion rises. The parallel increase of urinary citrate and calcium
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37 309 associated with higher urinary androgens mitigates the risk to develop kidney stones or
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39 310 nephrocalcinosis. While the mechanisms underlying the effect of androgens on tubular calcium
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41 311 handling have been studied, as outlined above, the impact of sex hormones on citrate transport
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43 312 in the proximal tubule have not been studied to our knowledge. As is the case for calcium,
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45 313 androgens may not only influence renal citrate reclamation in the proximal tubule but also alter
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47 314 circulating citrate levels (i.e. filtered load).

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49 315 In contrast to our results, no sex differences for the 24-hour excretion of urinary citrate were
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51 316 found in a Chicago cohort of 330 calcium oxalate stone formers [43] or in 807 kidney stone
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3 317 formers derived from three different US American cohorts of health professionals [44]. In our
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5 318 Bern cohort, higher 24-hour citrate excretion rates in men compared to women were found in
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7 319 both unadjusted and adjusted analyses, thus, apparent differences are not an effect of statistical
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9 320 methods. The 24-hour urine collections in the Bern cohort contain similar amounts of creatinine
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11 321 compared to the US cohorts, arguing against a systematic under- or over-collections of urine
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13 322 samples in one of the cohorts. Differences in urinary citrate excretion compared to the Chicago
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15 323 cohort may indicate different sex specific dietary habits in the rural population of Bern, and
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17 324 these sex specific differences may blur with an increasing degree of urbanization or even may
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19 325 disappear in metropolitan regions like Chicago. Furthermore, mean citrate excretion values in
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21 326 the US American cohorts of health professionals were considerably higher in both sexes
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23 327 compared to the cohorts from Chicago and Bern and may indicate a healthier, less meat-based
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25 328 diet in health professionals, or simply a better kidney function in the group of health
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27 329 professionals.

30
31 330 In contrast to urinary androgens, the situation is more complex with urinary estrogens. Our
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33 331 results suggest that urinary calcium excretion is positively associated with urinary 17β -estradiol
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35 332 and also with estriol in the setting of low urinary sulfate excretion (i.e. low dietary acid intake),
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37 333 whereas urinary oxalate excretion is not associated with any urinary estrogen. The association
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39 334 of urinary citrate excretion with estrogens is also diet dependent, being positive with 17β -
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41 335 estradiol (men and woman) and estriol (men only) in the setting of low urinary sodium excretion
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43 336 (i.e. low sodium intake or higher extrarenal sodium loss) and positive with estriol at a low
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45 337 sodium intake and in men only. 17β -estradiol is the predominant estrogen in premenopausal
46
47 338 women. Estriol has a weaker estrogenic activity, it is the predominant estrogen during
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49 339 pregnancy, but its role outside pregnancy has received little attention thus far [45]. The
50
51 340 interpretation of our results is somewhat complicated by the fact that estriol binds to estrogen
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53 341 receptors as a partial antagonist in the presence of 17β -estradiol [46], but it appears that 17β -

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3 342 estradiol and estriol can exert both stone promoting and stone inhibitory effects – stone
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5 343 promoting effects through a higher urinary calcium excretion and stone inhibitory effects
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7 344 through a higher urinary citrate excretion. Some of these effects seem to be influenceable by
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9 345 dietary habits and may therefore vary in the short to medium term. 17β -estradiol and estriol are
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11 346 synthesized from testosterone and androstenedione, respectively, by aromatase activity, which
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13 347 is reflected by the ratio testosterone/ 17β -estradiol (with a higher ratio corresponding to a lower
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15 348 enzyme activity). A significant interaction term with this ratio was found with age for the
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17 349 association with urinary oxalate, indicating an inverse association between oxalate excretion
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19 350 and aromatase activity in the elderly. The ratio testosterone/ 17β -estradiol also showed a
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21 351 significant interaction with NGIA for the association with urinary citrate, indicating an inverse
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23 352 association between citrate excretion and aromatase activity at a high NGIA. The significance
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25 353 of both findings remains unclear. Our results on estrogens are partially supported by previous
26
27 354 studies in postmenopausal calcium oxalate stone formers showing a higher 24-hour urinary
28
29 355 excretion of citrate and calcium, but not for oxalate, under ERT in one study [21], but a lower
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31 356 24-hour urinary calcium excretion and no difference for the excretion of oxalate and citrate
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33 357 under ERT in another study [22]. However, it should be noted that our study population differs
34
35 358 substantially from these studies, as we included 80% men, and only 39% of women were
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37 359 postmenopausal. In addition, differences in results may be explained by ERT related effects on
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39 360 bone or intestine, and even ERT related effects on the kidney may differ compared to the effects
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41 361 from endogenously derived estrogens at physiological levels. Results from animal studies and
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43 362 from *in vitro* data on the influence of estrogens on renal calcium handling and on potential
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45 363 underlying mechanism are conflicting [34].
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47 364 Our analysis was conducted with urinary steroid hormone profiles on a random outpatient diet
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49 365 and urinary steroid profile data from kidney stone formers on different dietary regimes were
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51 366 not available. In addition to demographic characteristics and kidney function, we therefore
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3 367 added three dietary factors to regression models in order to control for residual confounding
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5 368 due to individual dietary patterns among study participants. We have chosen NGIA as one
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7 369 dietary factor, for which a large influence on 24-hour urinary citrate excretion in stone-forming
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9 370 and healthy individuals is presumed [47]. In the present study, this influence expresses in a
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11 371 strong, positive and independent association between NGIA as explanatory co-variable and
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13 372 citrate as outcome variable, thereby corroborating previous findings [47]. We selected daily
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15 373 urinary sulfate and sodium excretion as two other potentially confounding dietary factors both
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17 374 known for their positive associations with daily urinary calcium excretion [48] and our results
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19 375 support the independency of both associations.
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22 376 The cross-sectional design is a limitation of this study and does not allow interfering with causal
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24 377 relationships. Another limitation is that no data about the phase of menstrual cycle are available.
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26 378 24-hour urines were collected outside the menstrual phase, and steroid hormone profiles from
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28 379 premenopausal women therefore derive from the follicular or luteal phase of the menstrual
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30 380 cycle. This may have an impact especially on urinary estrogens rather than on androgens or
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32 381 glucocorticoids as recently discussed by our group [32]. The phase of the menstrual cycle
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34 382 should be taken into account in future studies using urinary steroid hormone profiling.
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37 383 In summary, our findings suggest that androgens and estrogens influence urinary calcium and
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39 384 citrate excretion. In addition, we identified DHEA as a novel factor associated with urinary
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41 385 oxalate excretion in humans.
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3 387 **CONFLICT OF INTEREST STATEMENT**

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5 388 None of the authors have any conflicts of interest regarding this study. Results presented in this
6
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18 394 **AUTHORS' CONTRIBUTIONS**

19
20 395 DGF, GAM and NAD conceived and planned the study. BV provided project resources. DGF
21
22 396 and NAD recruited patients into the study. DGF, GAM, LS, CM, DL, and NAD conducted data
23
24 397 curation. NAD performed statistical analyses. NAD, GAM, and DGF wrote the manuscript with
25
26 398 input from all authors. All authors approved the final version of the manuscript.
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41 405 manuscript.
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46 407 **DATA AVAILABILITY STATEMENT**

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48 408 The data underlying this article will be shared on reasonable request to the corresponding
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50 409 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
Androstetriol	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						
Calcium oxalate (10-100%)	311	82%	76	76%	406	81%
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.

Explanatory variable	Unadjusted Model			Multivariable Model							
	Main explanatory variable			Main explanatory variable			Explanatory co-variable		Interaction main explanatory variable with co-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-Androstenediol	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: ¹Pregnenetriol/(THE+THF+5αTHF), ²(THA+THB+5αTHB)/(Androsterone+Etiocholanolone), ³(THA+THB+5αTHB)/(THE+THF+5αTHF), ⁴Pregnanediol/(17αOHpregnanolone+Pregnanetriol), ⁵Pregnenetriol/(DHEA+16αOHDHEA+Androstenediol+Androstenetriol), ⁶(17αOHpregnanolone+Pregnanetriol)/11βOHandrosterone, ⁷(17αOHpregnanolone+Pregnanetriol)/(THE+THF+5αTHF), ⁸Androsterone/Etiocholanolone, ⁹THF/5αTHF, ¹⁰THB/5αTHB, ¹¹Testosterone/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.

Explanatory variable	Unadjusted Model			Multivariable Model							
	N	β ; 95% CI	<i>p</i> -value	N	β ; 95% CI	<i>p</i> -value	Explanatory co-variable		Interaction main explanatory variable with co-variable		
							β ; 95% CI	<i>p</i> -value	β ; 95% CI	<i>p</i> -value	
DHEA	421	-0.018;-0.12-0.082	0.73	382	-0.11;-0.21 to -0.0050	0.040					
16 α -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					
Androstetriol	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					
5 α -DH-testosterone	451	0.11;0.013-0.20	0.025	410	0.012;-0.090-0.11	0.81					
5 α -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					
11 β -OH-androsterone	464	0.19;0.097-0.28	5.1E-05	420	0.078;-0.018-0.17	0.11					
Etiocolanolone	462	0.13;0.034-0.22	0.0079	419	0.051;-0.051-0.15	0.32					
17 β -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					
3 β -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					
Δ 4- and Δ 5-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
Δ 4-CYP17A1 ⁴	422	-0.16;-0.26 to -0.066	0.0010	386	-0.054;-0.15-0.045	0.28					
Δ 5-17,20-lyase ⁵	393	-0.0070;-0.11-0.096	0.89	355	-0.0093;-0.11-0.09	0.85					
Δ 4-17,20-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					
5 α -reductase ⁸	455	-0.028;-0.12-0.063	0.54	412	0.018;-0.074-0.11	0.70					
5 α -reductase ⁹	449	-0.063;-0.15-0.026	0.16	405	-0.019;-0.11-0.072	0.69					
5 α -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.

Explanatory variable	Unadjusted Model			Multivariable Model								
	Main explanatory variable			Main explanatory variable			Explanatory co-variable		Interaction main explanatory variable with co-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.

Figure 2

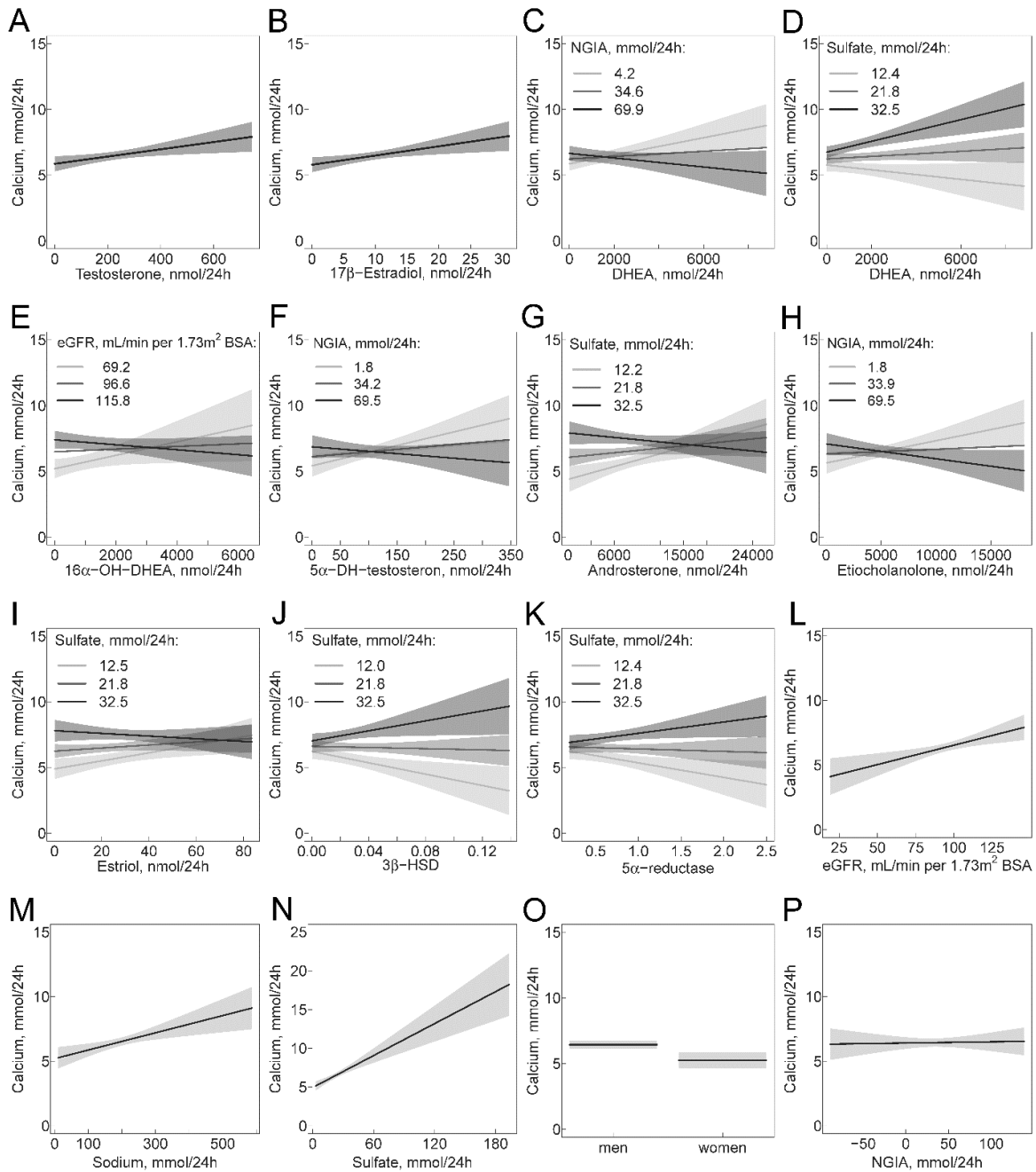


Figure 2. Association between urinary sex steroids, steroidogenic metabolite ratios and other predictors with 24-hour urinary calcium excretion. Adjusted regression models from Table 2 are visualized showing the relationship between the explanatory variable of interest on the x-axis and urinary calcium 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

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

Figure 3

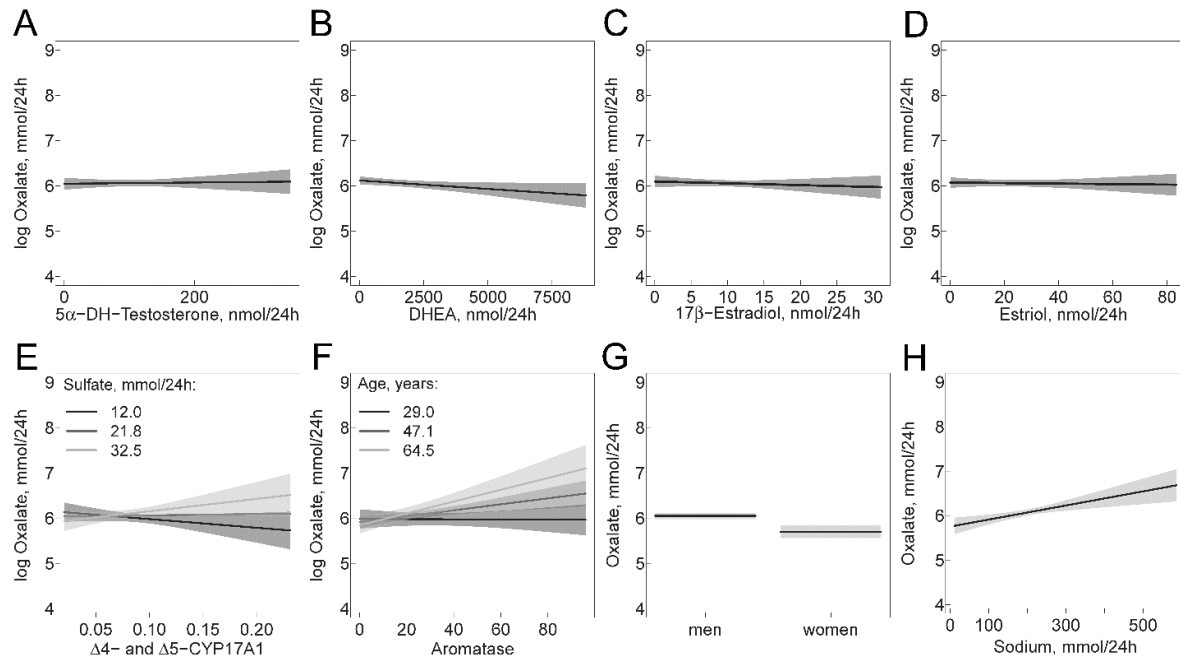


Figure 3. Association between urinary sex steroids, steroidogenic metabolite ratios and other predictors with 24-hour urinary oxalate excretion. Adjusted regression models from Table 3 are visualized showing the relationship between the explanatory variable of interest on the x-axis and urinary oxalate 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

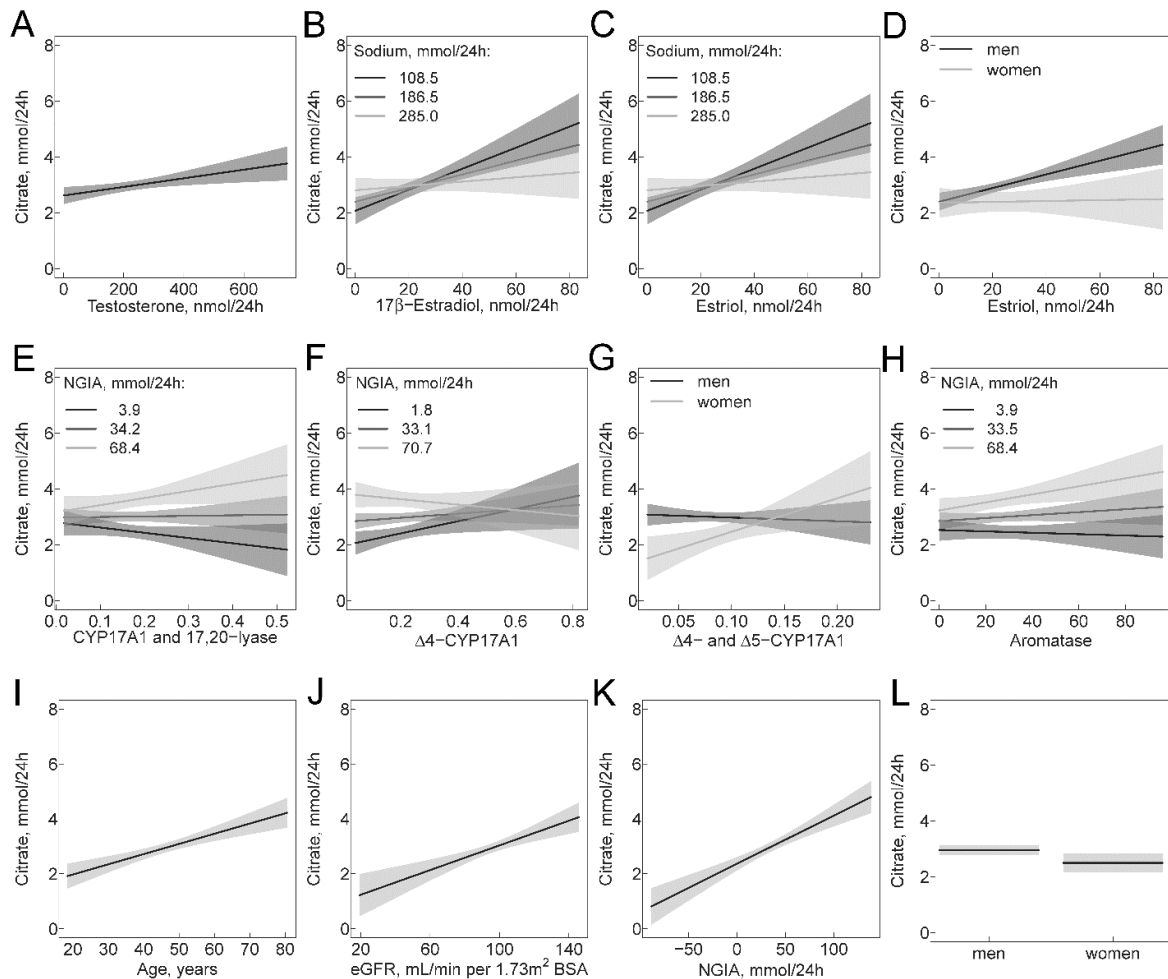


Figure 4. Association between urinary sex steroids, steroidogenic metabolite ratios and other predictors

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.