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Reference intervals for 24 laboratory parameters determined in 24-hour urine collections

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Abstract

Background: Reference intervals for many laboratory parameters determined in 24-h urine collections are either not publicly available or based on small numbers, not sex specific or not from a representative sample.
Methods: Osmolality and concentrations or enzymatic activities of sodium, potassium, chloride, glucose, creatinine, citrate, cortisol, pancreatic α-amylase, total protein, albumin, transferrin, immunoglobulin G, α_1-microglobulin, α_2-macroglobulin, as well as porphyrins and their precursors (δ-aminolevulinic acid and porphobilinogen) were determined in 241 24-h urine samples of a population-based cohort of asymptomatic adults (121 men and 120 women). For 16 of these 24 parameters creatinine-normalized ratios were calculated based on 24-h urine creatinine. The reference intervals for these parameters were calculated according to the CLSI C28-A3 statistical guidelines.
Results: By contrast to most published reference intervals, which do not stratify for sex, reference intervals of 12 of 24 laboratory parameters in 24-h urine collections and of eight of 16 parameters as creatinine-normalized ratios differed significantly between men and women. For six parameters calculated as 24-h urine excretion and four parameters calculated as creatinine-normalized ratios no reference intervals had been published before. For some parameters we found significant and relevant deviations from previously reported reference intervals, most notably for 24-h urine cortisol in women. Ten 24-h urine parameters showed weak or moderate sex-specific correlations with age.
Conclusions: By applying up-to-date analytical methods and clinical chemistry analyzers to 24-h urine collections from a large population-based cohort we provide as yet the most comprehensive set of sex-specific reference intervals calculated according to CLSI guidelines for parameters determined in 24-h urine collections.

Keywords: 24-h urine collection; cortisol; electrolytes; porphyrins; proteinuria; reference interval.

Introduction

Aliquots of 24-h urine collections are classical specimens for the determination of renally excreted endogenous substances which have a strong intra-individual variation in response to daytime, dietary habits and prandial state, physical activity, fluid uptake or urine volume. One major drawback of this specimen is the inaccuracy in sampling. Normalization of analyte concentrations in spot urine for urinary creatinine helped to overcome this problem for some but not all urinary biomarkers. Another major limitation for the diagnostic use of laboratory parameters determined in 24-h urine samples is the uncertainty of reference intervals. The cumbersome 24-h collection of urines makes it difficult to obtain sufficient numbers of samples required for state-of-the-art derivation of reference intervals.

Here we present sex-specific reference intervals for frequently requested laboratory parameters as well as porphyrins calculated according to recent CLSI guidelines [1] in 24-h urine collections of a large population-based cohort of 241 asymptomatic adult European individuals. These urine parameters were measured by the use of modern clinical chemistry analyzers and present day analytical methods.

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Materials and methods

Participants

The participants to this reference interval study are living in the canton of Zurich, Switzerland (see Table 1 for more information about the study population) and they form a sub-cohort of the Swiss Study on Salt intake, a representative random population-based cross-sectional study about the salt intake in Switzerland, who contributed 24-h urinary collections (for details see [2]). The study was approved by the Cantonal Ethics Committee Zurich, participation was voluntary and all participants gave written consent. Participants were instructed to collect urine in the container(s) without preservatives for 24 h according to the standard procedure [3]. Briefly, they were told to empty their bladder upon awakening in the morning, discard that sample and collect thereafter all voided urine portions including next morning's first urine in the container. The exact collection time was recorded by each participant. A blood sample was also obtained from each participant to this reference interval study to determine serum creatinine for the estimation of the glomerular filtration rate (GFR) [4]. Participants with an estimated GFR below 60 mL/min/1.73 m^2 or a 24-h urine collection of <600 mL were excluded from this study [5]. The final reference sample population consisted of 120 women (mean age: 43 y, range 19–82 y) and 121 men (mean age: 47 y, 20–89 y). Each 24-h urine collection was mixed and an aliquot of the mixed urine was centrifuged at 2000 *g* for 10 min. Measurements of electrolytes, osmolality, total protein, pancreatic α-amylase, glucose and creatinine were performed immediately after centrifugation of the urine samples. Aliquots of the centrifuged urine samples were stored at 2–8 °C before measurement of citrate, albumin, α_1-microglobulin, α_2-macroglobulin, transferrin, and immunoglobulin G (IgG) and at –20 °C before measurement of cortisol, porphyrins and their precursors.

Laboratory tests

The concentrations or enzymatic activities of the following parameters were determined by the use of a cobas 8000 analyzer as well as reagents from Roche Diagnostics (Rotkreuz, Switzerland); the analytical method employed is given in parenthesis: sodium, potassium and chloride (all by ion selective electrodes), creatinine (rate-blanked Jaffé reaction [6], total protein (turbidimetry with benzethonium chloride), pancreas α-amylase (colorimetric test with antibody inhibition of human salivary α-amylase) and glucose (hexokinase method), The concentrations of albumin, α_1-microglobulin, α_2-macroglobulin, transferrin, and IgG were determined by immuno-nephelometry on a BN ProSpec (Siemens Healthcare Diagnostics, Marburg, Germany). The concentrations of the porphyrins (uroporphyrin, hepta-, hexa- and pentacarboxy porphyrins, coproporphyrin I and III) and their precursors (δ-aminolevulinic acid and porphobilinogen) were determined by HPLC with fluorescence detection [7] and by ion-exchange chromatography with spectrophotometric detection after reaction with modified Ehrlich's reagent [8], respectively. Osmolality of the urine specimens was measured by freezing point depression (Advanced Instruments, Needham Heights, MA, USA). Citrate concentration was determined by an enzymatic UV-test (r-Biopharm, Darmstadt, Germany) on a Konelab 30i analyzer (Thermo Fisher Scientific, Vantaa, Finland). The cortisol concentration was determined by a chemiluminescent immunoassay without extraction of the analyte from the urine samples on an Access analyzer (Beckman Coulter, Nyon, Switzerland). For more details on the employed analytical methods see Table 2. Quality control material was analyzed in the same manner as the samples of the probands. The quality control was managed according to Swiss governmental regulations as defined by QUALAB (www.qualab.ch).

Data analysis

The reference intervals were determined for all participants as well as for women and men separately as the central 95% of the measurement results according to the CLSI C28-A3 statistical guidelines [1] using Reference Value Advisor [9], a set of macroinstructions for Microsoft Excel (Microsoft, Redmond, WA, USA) developed for this purpose. The 2.5th and the 97.5th percentiles of the distribution of the test results represent the lower and the upper reference limit, respectively, and were annotated with 90%

Table 1: Major characteristics of the study participants (percentage or mean±standard deviation).

Parameter	Women (n=120)	Men (n=121)
Caucasian ethnicity, %	97	97
Age, years	43±18	47±18
Body height, cm	167±6	178±7
Body weight, kg	64.7±9.3	82.4±13.5
BMI, kg/m^2	23.3±3.6	26.0±4.0
Diabetes mellitus, %	5	8
Hypertension, %	15	37
Oral anti-diabetic medication, %	0	3
Oral anti-hypertensive medication, %	7	18
Regular intake of medicaments, %	23	22
Current smoker, %	15	17
Urinary volume, mL/24 h	2205 (1600–2786)[a]	2040 (1440–2697)[a]

[a]For urinary volume the median is given together with the 1st and 3rd quartiles in parenthesis.

Table 2: Characteristics of the employed analytical methods.

Parameter	LLOQ	Linear range	Between-run imprecision (coefficient of variation)
Creatinine, mmol/L	0.375	0.375–55	≤4.2%
Citrate, mmol/L	0.07	0.07–3.00	≤9%
Potassium, mmol/L/24 h	1	1–100	≤2.1%
Sodium, mmol/L	10	10–250	≤2.7%
Chloride, mmol/L	10	10–250	≤2.3%
Glucose, mmol/L	0.1	0.1–41.6	≤1.0%
Cortisol, µg/L	4	4–60	≤5.8%
Pancreatic-α-amylase, U/L	3	3–1500	≤2.2%
Total protein, g/L	0.04	0.04–2.00	≤1.4%
Albumin, mg/L	2.2	2.2–68	≤3.2%
Transferrin, mg/L	2.2	2.2–35	≤2.5%
Immunoglobulin G, mg/L	3.6	3.6–58	≤2.7%
α_1-Microglobulin, mg/L	5.6	5.6–180	≤3.4%
α_2-Macroglobulin, mg/L	2.7	2.7–85	≤3.8%
δ-Aminolevulinic acid, µmol/L	3.0	3.0–500	≤6.2%
Porphobilinogen, µmol/L	2.5	2.5–300	≤2.6%
Uroporphyrin, nmol/L	0.5	0.5–1600	≤10%
Heptacarboxyporphyrin, nmol/L	0.2	0.2–1500	≤12%
Hexacarboxyporphyrin, nmol/L	0.2	0.2–1600	≤25%
Pentacarboxy porphyrin, nmol/L	0.4	0.4–1600	≤7.5%
Coproporphyrin I, nmol/L	0.7	0.7–1500	≤7.5%
Coproporphyrin III, nmol/L	1.4	1.4–1500	≤5%
Total porphyrins, nmol/L	0.5	0.5–1600	≤11%

LLOQ, lower limit of quantification.

confidence intervals. Outliers within the data sets were identified and removed with the Tukey test [10] after Box-Cox transformation [11] of the data to approximate a normal distribution. However, the data of some parameters was not normally distributed even after Box-Cox transformation (Andersen-Darling test). These data sets were visually inspected (box-whisker plots) to identify and remove extreme values. Box-Cox transformation was then applied to the rest of the data and if the transformed data was normally distributed the Tukey test was employed to identify and remove further outliers.

Quantities below the lower limit of quantitation (LLOQ) of the employed analytical method were assigned a value of LLOQ divided by the square root of two [12] for statistical data analysis. As recommended by the CLSI C28-A3 statistical guidelines [1], the reference intervals and the 90% confidence intervals for the reference interval limits were calculated by the use of the non-parametric rank method and the robust method of Horn and Pesce [13] for partitions with at least 120 and with <120 reference values, respectively. Statistical analysis was performed with Microsoft Excel (Microsoft, Redmond) and IBM SPSS Statistics for Windows (Version 21.0, Armonk, NY, IBM Corp). Visual inspection of the data, Spearman's, Pearson's correlation and least-square linear regression analysis were performed to infer age-dependency and the Mann-Whitney U-test to assess sex-dependency of the measured parameters. p-Values <0.05 were defined as statistically significant. Least-square regression analysis was performed only if all measurement results of a parameter were above the LLOQ of the employed analytical method and normally distributed.

Results

Table 3 shows the medians and reference intervals including the 90% confidence intervals for the lower and the upper reference limits for 24 laboratory parameters quantified in 24-h urine collections. Data are presented for the entire population as well as after stratification for men and women (see Table 3). Most of these data sets were not normally distributed. For half of the 24 parameters (creatinine, potassium, sodium, chloride, osmolality, cortisol, pancreatic α-amylase, δ-aminolevulinic acid, porphobilinogen, uroporphyrin, heptacarboxy porphyrin and coproporphyrin I) the upper and lower reference interval limits were higher in men than in women and the sex-dependency of the reference intervals was statistically significant.

Concentrations of cortisol, proteins, and porphyrins as well as their precursors in 24-h collections were also normalized for urinary creatinine concentrations (Table 4). In contrast to the higher absolute excreted amounts of the respective biomarkers in males, the creatinine-normalized urinary concentrations of uroporphyrin, hepta-, hexa- and pentacarboxy porphyrins did not differ in a statistically significant manner between men

Table 3: Reference intervals for 24-h urine parameters determined in this study and reported by others.

Parameter	All				Men				Women				p-Value
	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	
Creatinine, mmol/24 h													
This study	241	7 (5–8)	20 (20–22)	12	121	7 (6–10)	21 (20–22)	15	116	7 (7–8)	14 (14–15)	10	<0.0001
Published [14]						9	21			7	14		
Citrate, mmol/24 h													
This study	235	1.0 (0.6–1.1)	6.5 (6.0–7.2)	3.3	119	0.9 (0.8–1.1)	6.9 (6.2–7.5)	3.0	117	0.9 (0.6–1.2)	6.3 (5.9–6.7)	3.5	0.083
Published [15]						0.6	4.8			1.3	6.0		
Potassium, mmol/24 h													
This study	232	32 (27–37)	121 (104–123)	67	114	37 (33–41)	131 (121–132)	72	120	31 (21–35)	106 (94–121)	63	0.0008
Published [16]		25	125										
Sodium, mmol/24 h													
This study	234	43 (29–47)	257 (238–273)	135	121	47 (29–68)	326 (295–351)	159	118	38 (27–48)	217 (204–229)	121	<0.0001
Published [16]		40	220										
Chloride, mmol/24 h													
This study	236	46 (37–60)	256 (234–276)	137	119	58 (48–68)	289 (270–306)	160	118	48 (37–57)	207 (196–217)	124	<0.0001
Published [16]		110	250										
Glucose, mmol/24 h													
This study	233	0.1 (0.1–0.1)	0.6 (0.6–0.8)	0.3	113	0.1 (0.1–0.1)	0.7 (0.6–0.7)	0.3	120	0.1 (0.1–0.1)	0.7 (0.5–0.8)	0.2	<0.001
Published [17]		0.4	1.3										
Osmolality, mmol/kg H_2O													
This study	241	163 (118–185)	990 (877–1041)	388	121	165 (118–209)	1011 (963–1116)	463	117	146 (132–162)	743 (662–828)	327	<0.0001
Published [16]		300	900										
Cortisol, µg/24 h													
This study	238	80 (71–88)	344 (310–359)	156	119	88 (74–101)	351 (330–370)	209	118	77 (71–83)	199 (189–208)	132	<0.0001
Published[a]		58	403										
Pancreatic-α-amylase, U/24 h													
This study	228	52 (30–63)	274 (251–297)	139	119	51 (42–61)	327 (295–356)	154	115	50 (42–69)	252 (233–273)	125	0.003
Published[a]			305										
Total protein, g/24 h													
This study	236	0.04 (0.03–0.04)	0.15 (0.14–0.16)	0.8	120	0.05 (0.03–0.05)	0.16 (0.14–0.22)	0.08	120	0.04 (0.03–0.04)	0.17 (0.14–0.29)	0.07	0.0002
Published [18]			<0.14										
Albumin, mg/24 h													
This study	227	2 (1–3)	21 (17–27)	7	111	2 (2–3)	18 (16–21)	7	114	2 (2–3)	21 (18–24)	7	1.00
Consensus cut-off [19]			<30										
Transferrin, mg/24 h													
This study	241		<LLOQ		121		<LLOQ		120		<LLOQ		N.D.
Published	Not found												
Immunoglobulin G, mg/24 h													
This study	239	2.3 (2.0–2.5)	11.5 (10.4–13.0)	5.3	120	2.4 (2.0–2.6)	12.7 (10.4–14.7)	5.2	120	2.2 (2.0–2.6)	12.0 (9.9–13.0)	5.0	0.51
Published	Not found												

Table 3 (continued)

Parameter	All				Men				Women				p-Value
	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	
α_1-Microglobulin, mg/24 h													
This study	241	3.8 (3.7–4.3)	24.6 (22.2–26.7)	10.0	120	4.3 (3.8–5.1)	26.4 (24.3–26.7)	10.2	120	3.7 (3.4–4.2)	20.1 (16.6–21.7)	9.7	0.09
Published	Not found												
α_2-Macroglobulin, mg/24 h													
This study	239		<LLOQ		121		<LLOQ		120		<LLOQ		N.D.
Published	Not found												
δ-Aminolevulinic acid, µmol/24 h													
This study	236	4.7 (3.9–6.8)	36.0 (32.8–44.5)	18.9	116	4.5 (3.9–8.3)	35.1 (32.2–36.6)	20.3	116	5.3 (4.3–6.5)	30.8 (28.2–33.2)	16.7	<0.0001
Published [20]		0	50										
Porphobilinogen, µmol/24 h													
This study	229	3.3 (2.5–3.6)	11.5 (10.9–12.6)	6.8	113	3.2 (2.8–3.7)	12.3 (11.6–13.1)	7.3	116	2.9 (2.6–3.2)	10.7 (9.9–11.4)	6.2	0.0001
Published [20]		0	9										
Uroporphyrin, nmol/24 h													
This study	233	5.7 (4.0–6.5)	30.7 (26.7–32.4)	15.4	115	5.9 (4.3–7.5)	31.2 (29.4–33.0)	18.5	117	4.5 (3.6–5.5)	20.8 (19.7–21.8)	12.4	<0.0001
Published	Not found												
Heptacarboxyporphyrin, nmol/24 h													
This study	234	1.0 (0.7–1.4)	7.6 (7.4–8.5)	3.9	119	1.1 (0.6–1.6)	8.9 (8.3–9.5)	4.9	116	0.8 (0.5–1.0)	5.4 (5.1–5.7)	3.0	<0.0001
Published [20]		4	16										
Hexacarboxyporphyrin, nmol/24 h													
This study	239	0.1 (0.1–0.2)	3.1 (2.4–4.6)	0.7	119	0.1 (0.1–0.2)	2.8 (2.4–3.2)	0.8	117	0.1 (0.1–0.1)	2.6 (2.1–3.2)	0.5	<0.0001
Published [20]		0	2										
Pentacarboxyporphyrin, nmol/24 h													
This study	241	0.5 (0.3–0.7)	7.0 (6.5–7.9)	2.7	120	0.6 (0.3–1.0)	7.0 (6.5–7.9)	3.0	120	0.5 (0.3–0.7)	7.0 (5.5–7.3)	2.4	0.002
Published [20]		0	2										
Coproporphyrin I, nmol/24 h													
This study	238	8.7 (5.8–9.5)	62.9 (54.0–66.7)	24.0	120	10.4 (7.4–12.9)	67.1 (65.0–84.7)	28.2	120	7.9 (4.3–9.0)	45.2 (39.3–58.6)	20.4	<0.0001
Published [20]		5	90										
Coproporphyrin III, nmol/24 h													
This study	240	3.9 (2.4–10.3)	217 (196.7–277.1)	65.9	120	3.0 (1.5–8.2)	225.2 (197.1–279.5)	60.8	120	6.1 (2.4–11.8)	207.8 (172.4–277.1)	69.2	0.96
Published [20]		15	242										
Total porphyrins, nmol/24 h													
This study	240	24.7 (12.2–42.7)	312.9 (281.8–363.3)	122.6	120	20.2 (12.2–55.8)	321.1 (286.0–432.2)	128.7	120	24.7 (9.9–42.7)	311.9 (248.8–341.6)	121.7	0.019
Published	Not found												

90% CI, 90% confidence interval; LLOQ, lower limit of quantitation of the employed analytical method (see Table 2) as reference interval limit; LLRI, lower limit of the reference interval; m, median; n, number of study subjects, i.e. samples; N.D., not determined; ULRI, upper limit of the reference interval. p-Value of the Mann-Whitney U-test for the sex-dependency of the reference intervals. [a]Reference intervals provided by the diagnostic test manufacturer.

Table 4: Reference intervals for urine parameters after normalization for creatinine observed in this and previous studies.

Parameter	All				Men				Women				p-Value
	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	
Cortisol, µg/g creatinine													
This study	238	72 (58–76)	199 (182–223)	116	119	67 (62–73)	199 (186–212)	119	119	71 (66–76)	185 (172–199)	113	0.053
Published	Not found												
Total protein, mg/g creatinine													
This study	239	30 (26–31)	135 (120–158)	56	120	28 (20–31)	118 (106–124)	49	119	32 (30–35)	155 (134–178)	64	<0.0001
Published	Not found												
Albumin, mg/g creatinine													
This study	236	1.4 (1.0–1.6)	28.8 (17.3–33.1)	5.2	115	1.2 (1.0–1.4)	14.8 (12.6–17.4)	4.5	114	1.9 (1.6–2.2)	19.0 (16.0–22.1)	5.8	<0.0001
Published [21]			<46.9										
Consensus cut-off [19]			<30										
Transferrin, mg/g creatinine													
This study	241		<LLOQ		121		<LLOQ		120		<LLOQ		N.D.
Published [22]			<LOD										
Immunoglobulin G, mg/g creatinine													
This study	236	1.4 (1.3–1.5)	9.7 (8.8–10.2)	4.0	120	1.3 (0.9–1.5)	9.1 (6.8–9.9)	3.1	120	1.8 (1.5–2.3)	13.7 (10.0–21.6)	4.9	<0.0001
Published [21]			<8.8										
α_1-Microglobulin, mg/g creatinine													
This study	237	2.6 (2.4–3.0)	16.6 (15.4–17.5)	7.2	121	2.6 (1.9–2.8)	16.7 (15.2–20.5)	5.9	116	3.1 (2.6–3.6)	16.2 (14.9–17.5)	8.3	0.0002
Published [23]			<17										
α_2-Macroglobulin, mg/g creatinine													
This study	241		<LLOQ		121		<LLOQ		120		<LLOQ		N.D.
Published [24]			<7										
δ-Aminolevulinic acid, µmol/g creatinine													
This study	233	4.5 (3.0–5.4)	26.4 (25.4–27.6)	13.8	119	4.4 (3.5–5.5)	24.0 (22.4–25.5)	13.2	114	4.8 (3.5–5.9)	26.3 (24.7–27.9)	15.1	0.002
Published [20]		8.8	44.2										
Porphobilinogen, µmol/g creatinine													
This study	234	2.8 (2.4–3.1)	11.1 (10.1–11.9)	5.0	120	2.4 (0.8–2.9)	11.1 (10.0–13.1)	4.6	116	2.8 (2.6–3.0)	11.0 (10.1–12.0)		0.0004
Published [20]		0.9	7.1										
Uroporphyrin, nmol/g creatinine													
This study	233	5.2 (4.2–5.7)	19.5 (18.8–20.5)	11.6	120	5.3 (3.7–6.9)	22.8 (19.4–23.6)	11.3	119	4.6 (3.8–5.4)	20.9 (19.6–22.2)	11.9	0.56
Published	Not found												
Heptacarboxyporphyrin, nmol/g creatinine													
This study	230	0.9 (0.6–1.2)	5.2 (4.9–5.4)	2.9	114	1.2 (0.9–1.4)	4.8 (4.5–5.0)	2.9	118	0.8 (0.6–1.0)	5.5 (5.1–5.8)	2.8	0.33
Published [20]		0	11.5										
Hexacarboxyporphyrin, nmol/g creatinine													
This study	238	0.1 (0.1–0.1)	2.3 (1.9–3.1)	0.6	116	0.1 (0.1–0.1)	1.5 (1.3–1.6)	0.6	118	0.1 (0.1–0.1)	2.5 (2.0–3.0)	0.5	0.95
Published [20]		0	6.2										
Pentacarboxyporphyrin, nmol/g creatinine													
This study	240	0.4 (0.2–0.7)	5.1 (4.4–5.8)	2.0	120	0.3 (0.2–0.7)	4.1 (3.6–4.4)	1.9	120	0.4 (0.2–0.7)	5.8 (4.6–5.6)	2.2	0.06
Published [20]		0	8.8										

Table 4 (continued)

Parameter	All				Men			Women		p-Value
	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	n	LLRI (90% CI)	ULRI (90% CI)	m	
Coproporphyrin I, nmol/g creatinine													
This study	235	8.0 (2.1–10.4)	49.8 (45.6–53.4)	26.4	116	9.7 (8.2–11.5)	42.1 (39.6–44.4)	24.8	115	8.8 (6.4–11.2)	50.0 (47.0–53.1)	28.8	0.008
Published [20]		2.7	75.1										
Coproporphyrin III, nmol/g creatinine													
This study	241	3.8 (2.2–6.9)	164.9 (155.1–241.3)	49.3	120	3.3 (1.0–4.8)	155.0 (128.3–158.2)	38.8	119	5.7 (2.7–9.0)	192.7 (171.1–213.8)	62.7	0.002
Published [20]		15.0	230.0										
Total porphyrins, nmol/g creatinine													
This study	237	31.9 (18.0–36.5)	223.9 (212.4–246.9)	95.0	120	23.5 (8.2–36.5)	212.3 (191.6–228.0)	88.5	120	24.3 (8.8–41.3)	262.4 (223.7–325.6)	106.2	0.003
Published	Not found												

90% CI, 90%-confidence interval; LLOQ, lower limit of quantitation of the employed analytical method (see Table 2) as reference interval limit; LLRI, lower limit of the reference interval; LOD, limit of detection; m, median; n, number of study subjects, i.e. samples; N.D., not determined; ULRI, upper limit of the reference interval. p-Value of the Mann-Whitney U-test for the sex-dependency of the reference intervals. The conversion factor to obtain the amount of an analyte per mmol creatinine from the given amount per gram creatinine is 0.113.

and women. The upper reference interval limits of the creatinine-normalized ratios of total protein, albumin, IgG, δ-aminolevulinic acid, coproporphyrin I and III and total porphyrins were even higher in women than in men.

Several parameters in 24-h urine samples showed statistically significant correlations with age (Table 5). However, only the inverse correlations of creatinine (Figure 1A and B) and total porphyrins with age were seen in either sex. Other correlations with age were seen in one sex only, e.g. in males the positive correlations of citrate (Figure 1), total protein and α_1-microglobulin and the negative correlations of cortisol and coproporphyrin III. However, the negative correlations with age of sodium, chloride and coproporphyrin I were found for females only. To estimate the impact of age and hence the need of age-stratified reference intervals we calculated linear regression equations for normally distributed data sets. The absolute 24-h excretions showed mean changes per 10 years aging (Table 5) in the range of 3%–13% with respect to the median of the measurement results of these parameters.

Discussion

We here report the reference intervals of 24 laboratory parameters determined in 24-h urine collections from a population-based sample of 241 asymptomatic adults. The parameters were chosen because they are regularly requested by clinicians of our hospital and determined by applying present day analytical methods to modern clinical chemistry analyzers. The reference intervals of these parameters were calculated according to recent CLSI-guidelines.

For 18 parameters we found reference intervals either published or provided by the test manufacturers and the majority of these reference intervals were very similar to those of our study. For creatinine, citrate (data of women) and potassium the published reference interval limits were within the 90% confidence interval of our reference interval limits. They were obtained by using the same analytical methods that were employed in our study. However, despite the use of the same analytical system and methods, reference intervals of several other 24-h urine parameters differed in a statistically significant and clinically meaningful manner between our and previous reports. These differences became even more prominent by the stratification of reference intervals for sex.

Except for creatinine, citrate, coproporphyrin I and III, none of the previously published reference intervals

Table 5: Correlation and linear regression analysis results of 24-h urine parameters for age-dependency.

	Women						Men					
	n	r[a]	p-Value[b]	Slope[c]	Intercept[d]	Δ/10y[e]	n	r[a]	p-Value[b]	Slope[c]	Intercept[d]	Δ/10y[e]
Creatinine, mmol/24 h	116	−0.43	<0.0001	−0.04	12	−0.4	121	−0.33	0.0002	−0.06	18	−0.6
Citrate, mmol/24 h	117	0.11	0.22	0.009	3.1	0.09	119	0.43	<0.0001	0.04	1.5	0.4
Potassium, mmol/24 h	120	0.17	0.07	0.18	56	1.8	114	*0.18*	*0.06*			N.D.[f]
Sodium, mmol/24 h	118	−0.23	0.01	−0.58	149	−5.8	121	0.00	0.88	−0.05	160	0.5
Chloride, mmol/24 h	118	−0.19	0.04	−0.42	144	−4.2	119	0.04	0.66	0.13	154	1.3
Glucose, mmol/24 h	120	*0.03*	*0.71*			N.D.[g]	113	*0.08*	*0.42*			N.D.[g]
Osmolality, mmol/kg/H_2O	117	*−0.04*	*0.70*			N.D.[f]	121	*0.001*	*0.99*			N.D.[f]
Cortisol, µg/24 h	118	−0.07	0.45	−0.12	137	−1.2	119	−0.27	0.003	−1.00	261	−10
Pancreatic-α-amylase, U/24 h	115	*−0.14*	*0.13*			N.D.[f]	119	*0.10*	*0.30*			N.D.[f]
Total protein, g/24 h	120	*0.03*	*0.79*			N.D.[g]	120	*0.23*	*0.01*			N.D.[g]
Albumin, mg/24 h	114	*0.11*	*0.25*			N.D.[g]	111	*0.04*	*0.68*			N.D.[g]
Transferrin, mg/24 h			N.D.[h]			N.D.[g]			N.D.[h]			N.D.[g]
IgG, mg/24 h	120	*0.07*	*0.45*			N.D.[g]	120	*0.12*	*0.21*			N.D.[g]
α_1-Micro-globulin, mg/24 h	120	*0.10*	*0.27*			N.D.[g]	121	*0.34*	*0.0002*			N.D.[g]
α_2-Macroglo-bulin, mg/24 h			N.D.[h]			N.D.[g]			N.D.[h]			N.D.[g]
δ-Aminolevulinic acid, µmol/24 h	116	−0.15	0.11	−0.05	19.1	−0.5	116	0.00	0.86	−0.01	20.6	−0.1
Porphobilinogen, µmol/24 h	116	*0.08*	*0.39*			N.D.[f]	113	*0.04*	*0.67*			N.D.[f]
Uroporphyrin, nmol/24 h	117	−0.13	0.14	−0.03	17.8	−0.3	115	*−0.07*	*0.48*			N.D.[f]
Heptacarboxporphyrin, nmol/24 h	116	0.06	0.51	0.004	2.8	0.04	119	−0.13	0.15	−0.01	5.6	−0.1
Hexacarboxyporphyrin, nmol/24 h	117	*0.04*	*0.68*			N.D.[g]	119	*−0.04*	*0.66*			N.D.[g]
Pentacarboxy porphyrin, nmol/24 h	120	*−0.15*	*0.10*			N.D.[g]	120	*−0.2*	*0.20*			N.D.[g]
Coproporphyrin I, nmol/24 h	120	−0.19	0.04	−0.11	26.3	−1.1	120	−0.09	0.33	−0.08	34.2	−0.8
Coproporphyrin III, nmol/24 h	120	*−0.12*	*0.20*			N.D.[f]	120	*−0.20*	*0.03*			N.D.[f]
Total porphyrins, nmol/24 h	120	*−0.18*	*0.045*			N.D.[f]	120	*−0.18*	*0.04*			N.D.[f]

n, number of study subjects, i.e. samples. [a]Correlations and [b]p-Values were calculated according to Pearson except for numbers in italics which were calculated according to Spearman because of non-normal frequency distribution of the data. [c]Slope of the least-square linear regression analysis (units of the slope: units given in the first column of the table per year). [d]Intercept from least-square linear regression analysis (units of the intercept: units given in the first column of the table); [e]Δ/10y: mean change of the parameter in the 24-h urine in 10 years; N.D., not determined. Least-square linear regression analysis not performed because [f]The data is not normally distributed or [g]Because of the presence of measurement results below the LLOQ of the employed analytical method; [h]N.D., not determined, since more than 97.5% of the measurement results and hence the upper reference interval limit are below the LLOQ of the employed analytical method, independent of the participant's age.

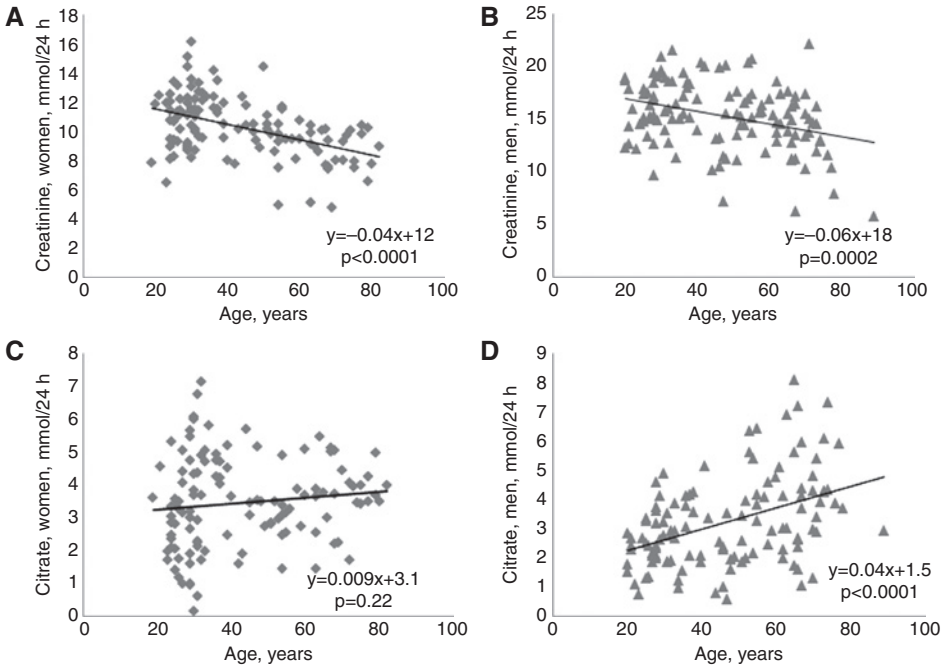

Figure 1: Scatter diagrams of 24-h urine creatinine for women (A), for men (B) and for 24-h urine citrate for women (C) and for men (D) versus age of the study participants.
The linear regression line, its slope, intercept and the statistical significance level are shown.

was sex-specific. By contrast in our study, for most of the parameters a statistically significant sex-dependency of the reference intervals was found, the lower and the upper reference limits being higher for men than for women. Although statistically significant, the sex-specific differences of reference intervals found for some parameters are of minor clinical relevance depending on the clinical indication for determining this parameter.

On the contrary, cortisol is a prominent example for discrepant reference intervals with clinical relevance. The 24-h urinary excretion of cortisol is recommended by endocrinological societies as the screening test for ruling out Cushing's syndrome [25] and should hence have a high diagnostic sensitivity. However, the test manufacturer provides a uniform reference interval with 14% and 103% higher upper reference limits (403 µg/24 h) than those determined by us in men (351 µg/24 h) and women (199 µg/24 h), respectively. A similar considerable sex difference in the upper reference limit was previously found in a study of 83 men and 104 women which used LC-MS/MS for the determination of urinary cortisol [26]. Especially for women, this discrepancy has a major impact on diagnostic sensitivity in ruling out hypercortisolism. The discrepancy between our sex-specific cortisol reference intervals and the uniform one of the test manufacturer determined in 140 apparently healthy adults is noteworthy, as the same analyzer and analytical method have been used.

Reference intervals of sodium concentrations were also concerned by important differences between the data provided either by our study or and the published uniform reference interval [16] cited by the test manufacturer (Table 3). The published upper reference limit of sodium excretion is 220 mmol/24 h [16] instead of 326 mmol/24 h for men in our study. Urinary electrolytes are known to show considerable intra- and inter-individual variations in response to changes in dietary intake. For sodium and potassium the intra-individual variation amounts to 31% and 24%, respectively [27]. The sodium excretion in our sample was determined at steady state conditions to reduce this biological variation and to generate representative data. Therefore, the higher median (135 mmol/24 h) and upper reference level probably reflect the higher than recommended salt intake [28] in the Swiss population, which however is in agreement with many other studies [29, 30].

Previous urinary porphyrin measurements using fractioned extractions [31] detected two fractions, the "uroporphyrin fraction" (uroporphyrin, heptacarboxy- and part of hexacarboxyporphyrins) and the "coproporphyrin fraction" (rest of hexacarboxy-, pentacarboxy- and coproporphyrins). Reference values based on this technique are outdated, as in the 1980s, HPLC with fluorescence detection was introduced, first as normal then later as reverse phase chromatography. The latter is technical standard

today. Published reference intervals of urinary porphyrins based on this HPLC method are available for children [32, 33] and for adults [7, 20, 34]. Table 3 shows the reference intervals of 24-h urine porphyrins for adults from the study of Hindmarsh et al. [20] which was performed with the largest sample of the aforementioned reference interval studies (96 adults). These reference intervals are in the same range as those of our study. However, it is worth mentioning that for porphyrin analysis a large inter-laboratory variation has been reported recently [35].

For 16 of the 24 parameters we also calculated creatinine-normalized excretions. Interestingly, this normalization either eliminated statistically significant differences of reference intervals between men and women or led to the occurrence of higher reference limits in women than in men. These changes reflect the significantly higher excretion of creatinine in men, which is the denominator of the ratios. Again we found some reference intervals being different from those published, e.g. for albumin, the porphyrins and their precursors. Some discrepant reference intervals may result from preanalytical differences in urine sampling. In clinical practice, specimens of second morning spot urines rather than 24-h urine collections are used for the determination of creatinine-normalized concentrations of urinary substances, e.g. for the differentiation and classification of proteinuria. In addition it is important to emphasize that clinical decision limits of some urinary parameters, e.g. albuminuria [19], have been defined by consensus of experts on the basis of clinical outcomes in epidemiological or clinical intervention studies rather than on the frequency distribution in the population. Similarly to another reference interval study for proteinuria marker proteins [21] the concentrations of these proteins were below the LLOQ of the analytical methods for many of the urine samples of our study. These concentrations were replaced by LLOQ divided by the root square of two [12] for statistical analysis as well as the calculation of reference intervals. The reference interval obtained for IgG using this approach is very close to that of the aforementioned study [21] with a similarly large 90% confidence interval for the upper reference interval limit. The concentrations of transferrin and of α2-macroglobulin were above the corresponding LLOQ in only three and only in one out of 241 samples, respectively, i.e. in <2.5% of the samples. Thus the 97.5th percentile, i.e. the upper reference interval limit, is below the LLOQ for the concentrations of these two proteins, as reported by another study on urinary transferrin [22].

For many parameters including creatinine, cortisol or citrate we found statistically significant correlations between urinary excretion and age. Nevertheless we did not define any age-dependent reference intervals for the following reasons: Most importantly, further stratification for age beyond sex would have yielded too small subgroups. Even a split by the median age of 36 and 47 years for women and men, respectively, would have generated groups of about 60 individuals and thereby large 90% confidence intervals for the reference interval limits. Furthermore, our linear regression analysis revealed that the mean 24-h urinary excretion of the analyzed parameters changed only by 3%–13% within 10 years of aging with respect to the median of the measurement results. This change is within the inter-assay imprecision of the methods used (Table 2). However, we cannot exclude that for particular age groups, e.g. seniors >70 years for which, however, only few data points are available in our study cohort, the mean relative change of 24-h urinary excretion of particular parameters per unit year aging is larger than expected by extrapolation.

Our reference interval study has several strengths and limitations. The major progress is the sample size which is larger than in most previous studies and allowed the determination of reference intervals with the non-parametric rank method recommended by the CLSI C28-A3 statistical guidelines [1]. As one limitation our population-based cohort, includes individuals with diabetes mellitus, hypertension, or regular intake of medicines (Table 1). These conditions may affect the urinary excretion of some parameters (e.g. albumin, total protein, glucose). To compensate for these uncertainties we used the Tukey test [10] and visual inspection of the data for the detection of outliers which were removed from the data set before calculation of reference intervals. Furthermore individuals with an estimated GFR <60 mL/min/1.73 m^2 were excluded from this study. Another weakness of this study is inherent with the sampling of 24-h urines in the population. Although the volunteers participating in our study were carefully instructed on the procedure of 24-h urine collection and only 24-h urine collections of more than 600 mL were included in this study, we cannot exclude incomplete specimen collection by some participants. As discussed before, reference intervals of some of the 16 parameters relative to urinary creatinine may differ from previously reported ones because we analyzed them in 24-h urine collections rather than in the conventional morning spot urine.

In conclusion, we here provide a clinically helpful resource of sex-specific reference intervals for 24 clinically relevant laboratory parameters determined in 24-h urine collections and for 16 parameters calculated as creatinine-normalized ratios.

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