Published online: 2019-06-07

Association Between Urinary Catecholamine Excretion and Urine Volume

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Key words
epinephrine, norepinephrine, dopamine, homovanillylmandelic acid, metanephrine, normetanephrine, vanillylmandelic acid, pheochromocytoma, paraganglioma

received 20.11.2018
accepted 14.05.2019

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DOI https://doi.org/10.1055/a-0926-3532
Published online: 7.6.2019
Horm Metab Res 2019; 51: 531–538
© Georg Thieme Verlag KG Stuttgart · New York
ISSN 0018-5043

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Supplementary Material for this article is available online at http://www.thieme-connect.de/products.

ABSTRACT

Several confounders must be considered in the evaluation of urinary catecholamine excretion. However, literature is contradictory about potential confounders. The aim of the present study was to assess correlations between catecholamine excretion and anthropometric or clinical parameters with special attention to urine volume. A total of 967 24-h urinary catecholamine measurements were performed in 593 patients for diagnostic purposes. The indication for urine examination was suspicion of secondary hypertension, phaeochromocytoma, or paraganglioma. From the patients examined, 57 % were females and 43 % were males. The patients' age ranged between 15 and 87 years with a median [Q1; Q3] of 51 [39; 62] years. Seventy-eight percent of the patients suffered from hypertension. Seventy percent of patients took one or more antihypertensive drugs. The most commonly used drugs were ACE inhibitors (43 %), while α-blockers (15 %) were the least used drugs. Urinary excretion was between 500 and 11 950 ml/24 h with a median of 2200 [1600; 2685] ml/24 h. The median body mass index (BMI) was 26.7 [24.0; 30.4] kg/m^2. The excretion of all catecholamines was greater in men than in women (all $p < 0.0001$). Epinephrine ($p = 0.0026$), dopamine ($p < 0.0001$), and metanephrine ($p = 0.0106$) excretion decreased with age. BMI was associated with urinary excretion of dopamine ($p < 0.0001$), norepinephrine ($p = 0.0026$), normetanephrine ($p < 0.0001$), and homovanillylmandelic acid (HVMA; $p = 0.0251$). Urine volume correlated with urinary dopamine ($p = 0.0127$), metanephrine ($p < 0.0001$), normetanephrine ($p = 0.0070$), and HVMA ($p < 0.0028$) excretion. In addition to the established associations between urinary catecholamine excretion and age, gender, and BMI in the present study, urinary catecholamine excretion correlated also with urine volume.

ABBREVIATIONS

ACE	Angiotensin-converting enzyme
AT1	Angiotensin II subtype 1 receptor
BC	Beta coefficient
BMI	Body mass index
CI	Confidence interval
COMT	Catechol *O*-methyltransferase
HPLC	High performance liquid chromatography
HVMA	Homovanillylmandelic acid
PCA	Pheochromocytoma
PGL	Paraganglioma
VMA	Vanillylmandelic acid

Introduction

Pheochromocytoma (PCA) or paraganglioma (PGL) are sporadic neuroendocrine tumors derived from cells of the adrenal medulla and paraganglia of the autonomic nervous system, respectively. The estimated incidence of PCA is approximately 0.8 per 100 000 person years [1]. Clinical presentations with adrenergic symptoms include episodic tachycardia, paroxysmal hypertension, anxiety, headaches, and sweating [2]. These tumors may be identified via incidental discovery on imaging or by selective screening of persons at risk. PGL originating from the sympathetic paravertebral ganglia of the thorax, abdomen or pelvis produce frequently catecholamines like norepinephrine and dopamine, while PGL arising from parasympathetic ganglia of the head and neck usually do not produce catecholamines. Chromaffin cells of adrenal tumors produce both epinephrine and norepinephrine, which are metabolized to metanephrine and normetanephrine, respectively. Given that these metabolites are constantly produced from tumor cells [3], determination of catecholamines and their metabolites homovanillylmandelic acid (HVMA), metanephrine, normetanephrine, and vanillylmandelic acid (VMA) plays an essential role in the diagnosis of catecholamine-producing tumors [4, 5]. In light of the high diagnostic validity of plasma normetanephrine and metanephrine in diagnosing PCA [6, 7], and, compared to urinary measurements, their more convenient determination and lack of inadvertent problems of accurate urine collection [8], the value of determination of urinary catecholamines and their metabolites has shortened in recent years.

In the diagnosis of pathological catecholamine production, the 24-h urine collection enables in particular determination of those hormones that are released in a pulsatile manner or attributed to a specific daily rhythm. However, these analyses can be influenced by several other factors like age [9], gender [10], body weight [9], smoking [11], eating habits, and the way of life [12], though the mentioned publications come to somehow contradictory results. The reasons for this may be that these papers differ significantly in terms of their study design, especially as regards sample composition and size as well as the use of exclusion criteria.

Previous work has shown an influence of urinary volume on the excretion of cortisone and cortisol [13, 14], whereas the possible influence of urine volume on catecholamine excretion has not been investigated in detail. In the present study comprising male and female patients of all weight and age groups, urinary excretion of catecholamines and catecholamine decomposition products was investigated. In order to avoid a bias resulting from factors known to influence catecholamine excretion, strict exclusion criteria such as pathological laboratory values indicating acute or chronic disease or the use of medications interfering with catecholamine measurement were applied. The aim of the present study was to detect potential correlations between catecholamine excretion and anthropometric or clinical parameters such as age, gender, body mass index, blood pressure and, in particular, urine volume.

Subjects and Methods

In the period from 2005 to 2008, urine was evaluated for catecholamines and catecholamine degradation products in 593 patients at the Medical Center Hospital at the University of Tübingen for diagnostic purposes. The indication for the urine examination was usually the suspicion of secondary hypertension or work-up of PCA or PGL. In addition to urinary catecholamine excretion, age, gender, body mass index, systolic and diastolic blood pressure, heart rate, diagnosis of arterial hypertension, and used anti-hypertensive drugs were documented.

Only patients with complete data sets and without exclusion criteria were included in the study. Since acute and chronic diseases may affect catecholamine excretion, patients with the following laboratory values were excluded from the study: creatinine > 1.5 mg/dl, C-reactive protein (CRP) > 5 mg/l, hemoglobin (Hb) < 8 g/dl, aspartate aminotransferase (AST) > 100 U/l, alanine aminotransferase (ALT) > 70 U/l, and gamma-glutamyltransferase (γ-GT) > 120 U/l. Patients already diagnosed with pheochromocytoma, as well as those with a suspected PCA (urinary catecholamines or their decomposition products ≥ double upper reference range) were also excluded from the study. Patients on medications that may interfere with catecholamine analysis were also excluded from the study. After application of these exclusion criteria, 593 patients with complete data sets remained in the study. In these patients, 967 24-hour urine samples were available for analysis. The numbers of urinary catecholamine measurements per visit are summarized in (**Supplementary ▶ Tables 1S** and **2S**).

Urine analysis

Urine was collected over a 24-h time period in an acidified urine container. To avoid sampling errors, the patients received written instructions for 24-h urine collection. The concentrations of epinephrine, norepinephrine, dopamine, HVMA, metanephrine, normetanephrine, and VMA were quantified by high performance liquid chromatography (HPLC) using an internal standard for calibration and subsequent quantification. Commercial kits were used for the analyses of all compounds of interest (Kits No 6000, 1000B, and 2020 Chromsystems, Grafelfingen, Germany). Before sample preparation, the pH of the urines were adjusted [15]. The catecholamines and their metabolites were separated from the urine matrix prior to chromatographic separation by solid phase extraction on an ion exchange matrix [16]. After sample pretreatment, an isocratic HPLC analysis was performed applying a flow rate of 1 ml/min and a total run time of 20 min. The electrochemical detection offers high sensitivity and selectivity due to the limited number of

chemical structures and functional groups accessible to a redox process at a given work potential [17]. The limit of detection (LOD) for epinephrine, norepinephrine and dopamine is 3 µg/l and the linearity range covers 3–1 000 µg/l. The LOD for HVMA and VMA is 0.5 mg/l and the linearity range is 75 mg/l and 50 mg/l, respectively. The LOD for metanephrine and normetanephrine is 5 µg/l and the linearity range reaches up to 5 000 µg/l. The intra-assay coefficients of variation (CV) for all detected compounds are below 5 % and the inter-assay CVs < 6 %.

The reference ranges for urinary catecholamines were provided by the company producing the HPLC-kit (Chromsystems). They are based on the publication of Ross and colleagues [18]. Ross et al. used data for reference range determination of urinary free catecholamine from a HPLC analysis with electrochemical detection of the 24-h urine of 106 patients. The patients were suspected of having a PCA, but the diagnosis was subsequently rejected. The reference ranges for urinary metanephrines were also provided by Chromsystems. They are based on the publication in the text book L. Thomas (ed.): Labor und Diagnose, chapter 32 [19].

Statistical analysis

Continuous and discrete variables were described using means and standard deviations or percentages, respectively. Due to repeated 24-h-urinary catecholamine measurements, linear mixed models with random intercepts were used to estimate associations between catecholamine measurements (as dependent variables) and independent variables, comprising age, gender, body mass index, systolic and diastolic blood pressure, as well as urinary volume. To account for the 7 hypotheses tested, a Bonferroni corrected α-level of < 0.05/7 = 0.007142857 was considered statistically significant. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

▶ **Table 1** Patients' characteristics (n = 593).

Variables	Values
Age (years)	51 [39; 62]
Gender (female/male)	338/255
Body mass index (kg/m^2)	26.7 [24.0; 30.4]
Systolic blood pressure (mmHg)	150 [130; 170]
Diastolic blood pressure (mmHg)	90 [80; 100]
Heart frequency (beats per minute)	71 [64; 79]
Arterial hypertension	459 (78 %)
Number of antihypertensive drugs	0–180 (30 %)
	1–147 (25 %)
	2–104 (18 %)
	3–76 (13 %)
	4–43 (7 %)
	5–34 (6 %)
	6–9 (1 %)
ACE inhibitors	256 (43 %)
Beta blockers	220 (37 %)
Calcium channel blockers	185 (31 %)
Diuretics	138 (23 %)
AT1 receptor antagonists	92 (16 %)
Alpha blockers	86 (15 %)
Urinary volume (ml)	2 200 [1600; 2685]

Data are shown as median [Q1; Q3] or numbers (percent).

Results

Patients' characteristics

▶ **Table 1** describes the characteristics of the 593 patients at the first visit, of which 57 % were females and 43 % were males. The age of the patients ranged between 15 and 87 years and was on average 51 years. Median body mass index was 26.7 kg/m^2, ranging between 17.2 to 55.0 kg/m^2. Seventy-eight percent of the patients examined suffered from arterial hypertension. Median systolic blood pressure was 150 mmHg and median diastolic blood pressure was 90 mmHg. Seventy percent of patients examined took one or more antihypertensive drugs, whereas 30 % of patients were without antihypertensive medication. The most commonly used drugs were angiotensin-converting enzyme (ACE) inhibitors (43 % of cases), while α-blockers (15 % of cases) were the least used drugs for treatment of hypertension. Other antihypertensive drugs used in the present study were beta blockers (37 % of cases), calcium channel blockers (31 % of cases), diuretics (23 % of cases), and angiotensin II subtype 1 receptor (AT1) antagonists (16 % of cases). Depending on the severity of hypertension, patients received 1-fold (25 % of patients), 2-fold (18 % of patients), 3-fold (13 % of patients), 4-fold (7 % of patients), 5-fold (6 % of patients), and up to 6-fold (1 % of patients) therapy. Urine volume ranged from 500 to 11950 ml/24 h with a median value of 2200 [1600; 2685] ml/24 h.

Epinephrine excretion

The linear mixed model analysis for epinephrine excretion showed a negative association between age and epinephrine excretion (p = 0.0026, beta coefficient (BC) –0.031, confidence interval (CI –0.052; –0.011)). Furthermore, an association between gender and epinephrine excretion was found with higher values in men (p = 0.0001, BC –1.89, CI –2.50; –1.29). While we found a clinically relevant association between systolic blood pressure and epinephrine excretion (p = 0.0507, BC 0.013, CI –0.00004; 0.027), there was no association between body mass index (p = 0.108), diastolic blood pressure (p = 0.874), urine volume (p = 0.632) and epinephrine excretion (▶ **Table 2**).

Dopamine excretion

▶ **Table 2** shows the linear mixed model analysis for dopamine excretion as the dependent variable. Age (p = 0.0001, BC –2.48, CI –2.97; –2.00) was negatively associated with dopamine excretion, whereas body mass index (p = 0.0001, BC 3.36, CI 2.06; 4.67) and urine volume (p = 0.0127, BC 0.0089, CI 0.0019; 0.016) correlated positively with dopamine excretion (▶ **Fig. 1a**), though the association between urine volume and dopamine excretion did not persist Bonferroni correction for multiple tests. Furthermore, an association between gender and dopamine excretion was found with

▶ **Table 2** Association between urinary dopamine, epinephrine, and norepinephrine excretion and age, gender, body mass index, blood pressure, and urinary volume.

Parameter	Estimate	p
Dopamine		
Age	−2.48 (−2.97; −2.00)	**<0.0001**
Gender	−51.3 (−65.9; −36.6)	**<0.0001**
Body mass index	3.36 (2.06; 4.67)	**<0.0001**
Systolic BP	−0.025 (−0.34; 0.29)	0.8758
Diastolic BP	0.35 (−0.26; 0.96)	0.2578
Urinary volume	0.0089 (0.0019; 0.016)	0.0127
Epinephrine		
Age	−0.031 (−0.052; −0.011)	**0.0026**
Gender	−1.89 (−2.50; −1.29)	**<0.0001**
Body mass index	−0.044 (−0.098; 0.0098)	0.1083
Systolic BP	0.013 (−0.00004; 0.027)	0.0507
Diastolic BP	0.0021 (−0.024; 0.028)	0.8740
Urinary volume	−0.00007 (−0.00038; 0.00023)	0.6329
Norepinephrine		
Age	0.002 (−0.11; 0.12)	0.9737
Gender	−11.18 (−14.70; −7.66)	**<0.0001**
Body mass index	0.49 (0.17; 0.81)	**0.0026**
Systolic BP	0.055 (−0.021; 0.13)	0.1566
Diastolic BP	0.090 (−0.058; 0.24)	0.2334
Urinary volume	0.00052 (−0.0012; 0.0022)	0.5416

BP: Blood pressure. p-Values in bold type were significant after correction for multiple tests.

higher values in men (p = 0.0001, BC −51.3, CI −65.9; −36.6). In contrast, there was no association between systolic blood pressure or diastolic blood pressure and dopamine excretion (both p > 0.05).

Norepinephrine excretion

As shown in ▶ **Table 2**, the linear mixed model analysis for norepinephrine excretion as the dependent variable showed a positive association between body mass index (p = 0.0026, BC 0.49, CI 0.17; 0.81) and norepinephrine excretion. Additionally, an association between gender and norepinephrine excretion was found with higher values in men (p = 0.001, BC −11.18, CI −14.70; −7.66). For the other independent variables like age, systolic blood pressure, diastolic blood pressure and urine volume, however, no correlation with norepinephrine excretion was found (all p > 0.05).

Homovanillylmandelic acid (HVMA) excretion

The linear mixed model analysis for HVMA excretion as the dependent variable showed a clinically relevant negative association with age (p = 0.0631, BC −0.019, CI −0.039; −0.001) and a positive association with body mass index (p = 0.0251, BC 0.059, CI 0.0074; 0.11) and urine volume (p = 0.0028, BC 0.00043, CI 0.00015; 0.00072) (▶ **Fig. 1b**). Moreover, an association between gender and HVMA excretion was found with higher values in men (p = 0.0484, BC 0.059, CI 0.0074; 0.11), though the associations of HVMA excretion with age and gender did not persist Bonferroni correction for multiple tests. The other independent variables like systolic and diastolic blood pressure revealed no associations with HVMA excretion (all p > 0.05; ▶ **Table 3**).

Metanephrine excretion

The linear mixed model analysis for metanephrine excretion showed a negative association with age (p = 0.0106, BC −0.39, CI −0.69; 0.091) that did not persist Bonferroni correction for multiple tests and a positive association with urine volume (p = 0.0001, BC 0.01110, CI 0.0068; 0.015) (▶ **Fig. 1c**). In addition, an association between gender and metanephrine excretion was found with higher values in men (p = 0.0001, BC −32.6, CI −41.5; −23.7). The remaining independent variables systolic and diastolic blood pressure as well as body mass index showed no associations with metanephrine excretion (all p > 0.05; ▶ **Table 3**).

Normetanephrine excretion

The linear mixed model analysis for normetanephrine excretion as the dependent variable showed a clinically relevant positive association with age (p = 0.0517, BC 0.75, CI −0.0057; 1.51) and positive associations with body mass index (p = 0.0001, BC 5.31, CI 3.34; 7.29), systolic blood pressure (p = 0.0136, BC 0.5977, CI 0.12; 1.07), and urine volume (p = 0.007, BC 0.015, CI 0.0042; 0.026), though the association between normetanephrine excretion and systolic blood pressure did not persist Bonferroni correction for multiple tests (▶ **Fig. 1d**). In addition, an association between gender and normetanephrine excretion was found with higher values in men (p = 0.0001, BC −47.0, CI −69.5; 24.5). There was no association between normetanephrine excretion and diastolic blood pressure (p > 0.05; ▶ **Table 3**).

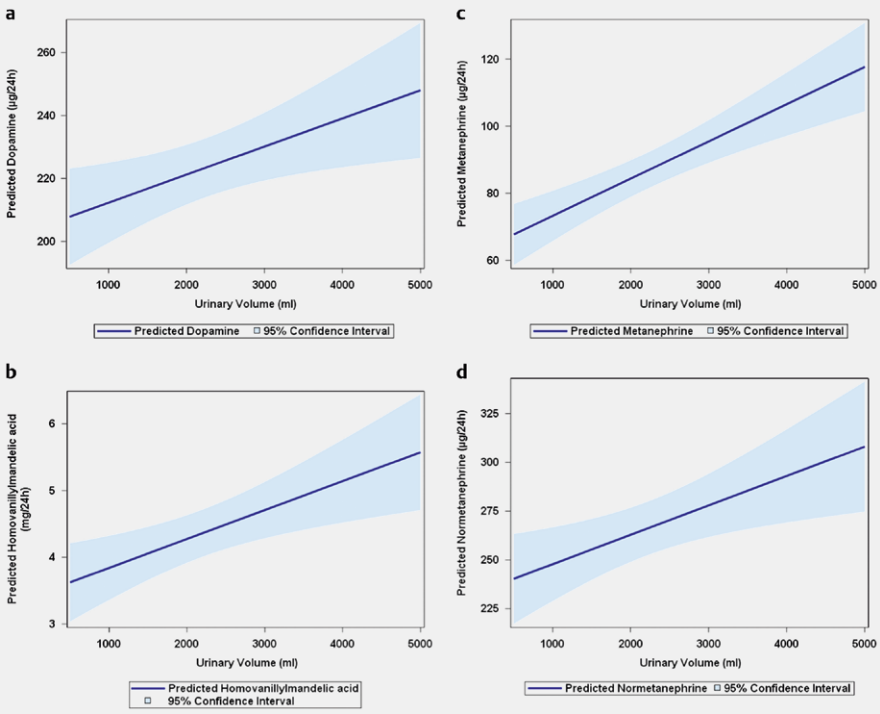
Vanillylmandelic acid (VMA) excretion

The linear mixed model analysis for VMA excretion as the dependent variable showed a clinically relevant association with body mass index (p = 0.0656, BC 0.024, CI −0.0015; 0.049). Furthermore, an association between gender and VMA excretion was found with higher values in men (p = 0.0001, BC −0.74, CI −1.03; −0.45). For the other independent variables age, urine volume, systolic and diastolic blood pressure, however, no association with VMA excretion was found (all p > 0.05; ▶ **Table 3**).

In order to account for the antihypertensive medication as a potential confounding factor, we performed an additional sensitive analysis. Also after including the number of antihypertensive drugs taken by the patients as well as its interaction with age in the mixed linear models the results were not changed relevantly (**Supplementary** ▶ **Tables 3S** and **4S**).

Discussion

In the present study, the influence of anthropometric and clinical parameters comprising age, gender, body mass index, blood pressure and urine volume on urinary catecholamine excretion was investigated.



▶ **Fig. 1** Predepicted urinary **a** dopamine, **b** homovanillylmandelic acid (HVMA), **c** metanephrine, and **d** normetanephrine excretion as a function of urine volume.

In contrast to previous studies, not only the catecholamines epinephrine, dopamine and norepinephrine were determined in 24-h urine samples, but also their metabolites HVMA, metanephrine, normetanephrine, and VMA.

Urine volume was positively associated with the excretion of dopamine, HVMA, metanephrine, and normetanephrine. In contrast, no association was found between urine volume and epinephrine, norepinephrine and VMA excretion. In agreement with our findings, Kawano et al., reported a positive association between 24-h urine volume and urinary dopamine excretion and no association between urine volume and epinephrine or norepinephrine excretion [20]. In contrast, early studies on catecholamine excretion in 24-h urine did not reveal a direct relationship between catecholamine excretion and urine volume [21–23]. Various reasons may have contributed to these contradictory results. In particular, the method used to determine urinary catecholamines has to be considered. For instance, a comparison between an immunological assay using monoclonal antibodies and an HPLC method showed only poor correlations between both methods [15]. Other factors which may influence urinary catecholamine excretion comprise smoking [9], environmental [10] and dietary factors. A higher salt intake increases urinary catecholamine excretion [24], whereas a low protein intake reduces the urinary excretion of dopamine [25]. None of the aforementioned studies has been controlled for these potential confounders.

In the present study, gender differences were found for the excretion of all catecholamines, with higher levels in men, in agreement with 2 previous studies showing greater excretion of all 3 catecholamines in men than in women [10, 26]. However, according to the results of the present study, this applies not only to epinephrine, dopamine and norepinephrine, but also to their metabolites HVMA, metanephrine, normetanephrine and VMA which have not been tested by the 2 previous studies. There are further differences between the present work and the studies by Gerlo et al. and Lakatua et al. In the study by Gerlo et al., the number of included individuals was larger with 956 patients. However, the exclusion criteria were less strict. For instance, patients with PCA were not excluded. As a measure of the severity of concomitant disease only the criterion of intensive care treatment was used. In contrast, in the present work, several pathological laboratory parameters indicating acute or chronic disease were applied. In the study by Lakatua et al., the sample size was smaller with 340 patients, comprising both children and adults. Exclusion criteria were not taken into account.

While the age-related differences in catecholamine excretion in children have been well documented by several studies [27–29], there is just little work on the same issue in adults [10, 28, 30]. Our findings showing a decrease of epinephrine and dopamine excretion with age and no age dependence for norepinephrine excretion are in line with previous studies [26, 29, 30]. In addition, HVMA excretion showed a clinically relevant negative association with age and metanephrine excretion was negatively associated with age, whereas normetanephrine excretion demonstrated a clinically relevant positive association with age. There was no association between age and VMA excretion. Urinary excretion of dopamine is predominantly formed by decarboxylation of plasma l-dopa in the kidney [31, 32]. In rats, an age-depend-

▶ **Table 3** Association between urinary metanephrine, normetanephrine, HVMA and VMA excretion and age, gender, body mass index, blood pressure, and urinary volume.

Parameter	Estimate	p
Metanephrine		
Age	−0.39 (−0.69; 0.091)	0.0106
Gender	−32.6 (−41.5; −23.7)	**<0.0001**
Body mass index	−0.70 (−1.47; 0.08)	0.0788
Systolic BP	0.15 (−0.042; 0.33)	0.1269
Diastolic BP	−0.006 (−0.36; 0.35)	0.9732
Urinary volume	0.011 (0.0068; 0.015)	**<0.0001**
Normetanephrine		
Age	0.75 (−0.0057; 1.51)	0.0517
Gender	−47.0 (−69.5; 24.5)	**<0.0001**
Body mass index	5.31 (3.34; 7.29)	**<0.0001**
Systolic BP	0.60 (0.12; 1.07)	0.0136
Diastolic BP	−0.077 (−0.97; 0.81)	0.8646
Urinary volume	0.015 (0.0042; 0.026)	**0.0070**
Homovanillylmandelic acid		
Age	−0.019 (−0.039; −0.001)	0.0631
Gender	−0.61 (−1.21; −0.0043)	0.0484
Body mass index	0.059 (0.0074; 0.11)	0.0251
Systolic BP	0.0016 (−0.013; 0.016)	0.8286
Diastolic BP	0.0043 (−0.023; 0.032)	0.7572
Urinary volume	0.00043 (0.00015; 0.00072)	**0.0028**
Vanillylmandelic acid		
Age	0.0053 (−0.0045; 0.015)	0.2838
Gender	−0.74 (−1.03; −0.45)	**<0.0001**
Body mass index	0.024 (−0.0015; 0.049)	0.0656
Systolic BP	0.0049 (−0.0017; 0.012)	0.1476
Diastolic BP	−0.0036 (−0.016; 0.0087)	0.5631
Urinary volume	0.00049 (−0.00009; 0.00019)	0.4953

BP: Blood pressure. p-Values in bold type were significant after correction for multiple tests.

ent rise in renal dopamine tissue levels has been described, which has been explained by an increasing synthesis [33, 34]. Though the precise cause of the higher renal dopamine production in older age is unknown, it may be a feedback mechanism protecting against a diminishing function of the aging kidney [35]. However, despite increased dopamine tissue levels, dopamine excretion is reduced in older rats [33]. This result is consistent with the reduced dopamine excretion in the elderly shown in the present work.

In patients with chronic renal parenchymal damage, daily urinary excretion of levodopa oder L-DOPA, free dopamine, and dopamine metabolites correlates negatively with the degree of renal impairment [36]. The decreased dopamine excretion in renal insufficiency [37] may result from reduced activity of the renal dopaminergic system in chronic renal parenchymal disease [38]. The underlying pathophysiological mechanism is the progressive loss of tubular units which have the ability to synthesize dopamine from levodopa oder L-DOPA [39].

These findings are in line with our results. Though moderate to severe chronic kidney disease was an exclusion criterion, kidney function as assessed by serum creatinine levels correlated negatively with urinary dopamine excretion when adding serum creatinine to the linear mixed model analysis ($p < 0.0001$). On the other hand, in particular medications may influence dopamine secretion and may lead to increased levels. Drugs contributing to elevated dopamine levels comprise intravenous dopamine, levodopa oder L-DOPA, methyldopa, clozapine, antidepressants and metoclopramide and must, therefore, be taken into account when interpreting the data [40, 41].

In agreement with the present study in humans which found no age dependence for norepinephrine excretion, in a previous study in rodents, norepinephrine excretion was similar in 3-month-old and 12-month-old rats [33]. In contrast to neural cells, renal proximal tubule cells do not express dopamine hydroxylase, therefore renal dopamine is not converted to norepinephrine [42–44]. Given that norepinephrine is not produced in the kidney, decreasing renal function with age has no effect on norepinephrine excretion. However, investigations in mice showed a reduced synthesis of all catecholamines with increasing age in the adrenal glands [45]. In addition, mRNA expression levels of enzymes necessary for catecholamine synthesis, such as tyrosine hydroxylase, dopa decarboxylase, dopamine beta-hydroxylase, and phenylethanolamine N-methyltransferase, were diminished [45]. The cause of the constant norepinephrine excretion also in advanced age may be that only a small part of norepinephrine is produced in the adrenal medulla [27].

Ito et al. found positive correlations between systolic blood pressure and urinary excretion of dopamine, epinephrine, and norepinephrine [46], whereas diastolic blood pressure did not correlate with excretion of catecholamine metabolites [47]. In contrast, we did not detect associations between systolic blood pressure and urinary excretion of catecholamines or their metabolites, except for a clinically relevant association between systolic blood pressure and epinephrine excretion. In a previous dietary intervention study in rats, urinary catecholamines paralleled the changes in systolic and diastolic blood pressure, decreasing during calorie restriction and increasing during refeeding [48]. However, only epinephrine, norepinephrine and dopamine were studied. In a pilot study comprising 20 patients, urinary normetanephrine excretion correlated positively with systolic blood pressure, whereas urinary metanephrine excretion showed no correlation with systolic blood pressure [49]. The authors hypothesized that the sympathetic nervous system activity might affect systolic blood pressure variability. These results partially contradict our findings, except for the correlation between normetanephrine excretion and systolic blood pressure. This discrepancy may be due to the high percentage of patients (70 % of cases) taking at least one antihypertensive drug in the present study which might have attenuated the association between catecholamine excretion and systolic blood pressure.

With regard to a possible association between body weight and degree of activity of the sympathetic nervous system or catecholamine excretion, previous clinical studies have yielded different results. Some studies showed a positive correlation [16, 50–54], others showed an inverse correlation [55, 56], and yet other studies showed no correla-

tion [9, 28, 57]. The present study revealed positive correlations between norepinephrine and dopamine excretion and BMI and no correlation between epinephrine excretion and BMI. With the exception of metanephrine and VMA, which showed clinically relevant positive correlations, analyses of all other metabolites depicted significantly positive associations with body mass. Different reasons may have caused the discrepancies between our results and previous studies. First, various subtypes of obesity may exist which are not reflected by BMI alone [58]. Second, the samples of each study differ in terms of size and application of exclusion criteria. Third, excretion of urinary catecholamines was measured by different assays, with measurement of the activity of the sympathetic nervous system being difficult in general. The measurements of norepinephrine levels in plasma and urine as indices of the activity of the sympathetic nervous system are of limited sensitivity, since norepinephrine is not a circulating hormone, but is released locally by sympathetic nerve endings. Thus, moderate changes in sympathetic nervous system activity may not be detected in plasma or urine [59]. Because norepinephrine can also be produced in the adrenal glands in certain cases, it is difficult to distinguish whether norepinephrine comes from the sympathetic nervous system or adrenal nerve endings [59]. The results of the present work on the correlation between body mass and catecholamine excretion, however, are in line with previous studies showing that dietary weight loss as well as anorexia nervosa has a significant impact on catecholamine excretion [54, 60, 61]. Several approaches may serve to explain the increased catecholamine excretion in obese people: on the one hand hyperinsulinemia which is frequently found in obesity leads to a stimulation of the sympathetic nervous system [62], on the other hand, increased leptin levels have similar effects [63]. In obesity, leptin is increasingly released from fat cells in order to inhibit appetite-stimulating hormones. However, over time leptin resistance may develop resulting in attenuation of the leptin signaling. Furthermore, studies on the excretion of norepinephrine in obese humans showed a regional hyperactivity of the sympathetic nervous system in the kidneys [64].

Despite the strengths of our study, including the large number of measurements, the rigorous exclusion criteria, and the consideration of several potential confounding factors, there are also some limitations, such as the lacking correction of urinary catecholamines and their decomposition products by creatinine measurements. However, in order to avoid sampling errors, the patients received written instructions for 24-h urine collections.

Conclusion

In addition to the well-established associations between urinary catecholamine excretion and age and gender and the more controversially discussed associations between urinary catecholamines and body mass index and blood pressure, in the present study, urinary catecholamine excretion were also associated with urine volume. This has to be considered as a potential influencing factor in the evaluation of urinary excretion levels of catecholamines and their respective degradation products.

Conflict of Interest

The authors declare that they have no conflict of interest.

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