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Unilateral and Bilateral Adrenalectomy for Pheochromocytoma Requires Adjustment of Urinary and Plasma Metanephrine Reference Ranges

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Context: Follow-up after adrenalectomy for pheochromocytoma is recommended because of a recurrence risk. During follow-up, plasma and/or urinary metanephrine (MN) and normetanephrine (NMN) are interpreted using reference ranges obtained in healthy subjects.

Objective: Because adrenalectomy may decrease epinephrine production, we compared MN and NMN concentrations in patients after adrenalectomy to concentrations in a healthy reference population.

Design: A single-center cohort study was performed in pheochromocytoma patients after adrenalectomy between 1980 and 2011.

Subjects: Seventy patients after unilateral and 24 after bilateral adrenalectomy were included.

Main Outcome Measures: Plasma-free and urinary-deconjugated MN and NMN determined at 3 to 6 months and annually until 5 years after adrenalectomy were compared with concentrations in a reference population. Data are presented in median (interquartile range).

Results: Urinary and plasma MN concentrations 3 to 6 months after unilateral adrenalectomy were lower compared with the reference population (39 [31–53] μmol/mol creatinine and 0.14 [0.09–0.18] nmol/L vs 61 [49–74] μmol/mol creatinine and 0.18 [0.13–0.23] nmol/L, respectively, both $P < .05$). Urinary MN after bilateral adrenalectomy was reduced even further (7 [1–22] μmol/mol creatinine; $P < .05$). Urinary and plasma NMN were higher after unilateral adrenalectomy (151 [117–189] μmol/mol creatinine and 0.78 [0.59–1.00] nmol/L vs 114 [98–176] μmol/mol creatinine and 0.53 [0.41–0.70] nmol/L; both $P < .05$). Urinary NMN after bilateral adrenalectomy was higher (177 [106–238] μmol/mol creatinine; $P < .05$). Changes in urinary and plasma MNs persisted during follow-up.

Conclusion: Concentrations of MN are decreased, whereas NMN concentrations are increased after unilateral and bilateral adrenalectomy. Adjusted reference values for MN and NMN are needed in the postsurgical follow-up of pheochromocytoma patients. ***(J Clin Endocrinol Metab* 98: 1076–1083, 2013)**

Pheochromocytomas are rare catecholamine-producing tumors, derived from adrenal chromaffin tissue (1). Pheochromocytomas occur sporadically or as part of several hereditary tumor syndromes, such as multiple endocrine neoplasia (MEN) type 2, von Hippel-Lindau syndrome, neurofibromatosis type 1 and in the context of succinate dehydrogenase (*SDH*) mutations (2). In pheochromocytoma patients, the demonstration of excessive production of

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Abbreviations: BP, blood pressure; DOPA, dihydroxyphenylalanine; LC-MS/MS, liquid chromatography tandem mass spectrometry; LRL, lower reference limit; MEN, multiple endocrine neoplasia; MIBG, metaiodobenzylguanidine; MN, metanephrine; NMN, normetanephrine; PET, positron emission tomography; PNMT, phenylethanolamine-*N*-methyltransferase; URL, upper reference limit.

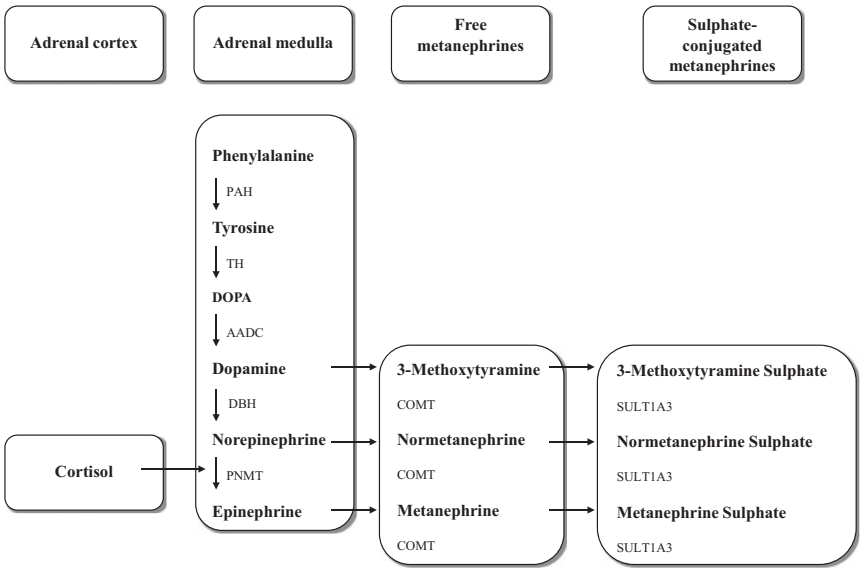

Figure 1. Schematic representation of catecholamine synthesis and metabolism pathway inside the adrenal gland. Dopamine, norepinephrine, and epinephrine are synthesized inside the adrenal medulla. DOPA is converted to dopamine by the aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28) enzyme. Dopamine is converted by the dopamine β-hydroxylase (DBH; EC 1.14.17.1) enzyme to norepinephrine, which is finally converted to epinephrine by the PNMT enzyme. PNMT is stimulated by cortisol from the adrenal cortex. The catecholamines are metabolized by the catechol-*O*-methyltransferase (COMT; EC 2.1.1.6) enzyme and can be measured as 3-methoxytyramine (3-MT), NMN, and MN in plasma. The MNs are sulfated by the sulfotransferase isoenzyme 1A3 (SULT1A3; EC 2.8.2.1). The sulfated (conjugated) MNs and the free (unconjugated) MNs are measured in urine. Abbreviations: PAH, phenylalanine hydroxylase (EC 1.14.16.1); TH, tyrosine hydroxylase (EC 1.14.16.2).

catecholamines and their 3-*O*-methylated metabolites, metanephrine (MN) and normetanephrine (NMN), constitutes the cornerstone of biochemical diagnosis (3–5). In healthy individuals, the adrenal medulla together with the sympathetic nervous system represent the main sources of catecholamine production and metabolism (6) (Figure 1). Cortisol from the adrenal cortex enhances the activity of phenylethanolamine-*N*-methyltransferase (PNMT; EC 2.1.1.28) (7, 8). Because the enzymatic conversion of norepinephrine to epinephrine by PNMT is cortisol-dependent, the adrenal medulla is main source of MN measured in urine and/or plasma (6, 9).

Resection of a pheochromocytoma through adrenalectomy is indicated to prevent potentially fatal complications due to catecholamine excess (10, 11). Postoperatively, periodic measurement of plasma and/or urinary MN and NMN is performed to monitor for recurrent disease. Follow-up is recommended 2 to 3 months postoperatively, annually during the first 5 years, and once every 2 years thereafter (12). During long-term follow-up, 6%–16% of tumors recur within 10 years postoperatively, but tumor recurrences have been described even after 15 years (13–15). Follow-up is especially important for patients with hereditary tumor syndromes, because these individuals have an increased risk for developing a pheochromocytoma in the contralateral adrenal gland (13, 14).

In routine clinical practice, the concentrations of urinary and/or plasma MN and NMN found in patients after adrenalectomy are interpreted by using reference intervals established in healthy subjects. However, unilateral or bilateral adrenalectomy is expected to be followed by a decrease of epinephrine and, consequently, lower plasma and urinary MN concentrations. Use of incorrect reference intervals may interfere with early detection of tumor recurrence, eg, when the associated rise in MN concentration occurs within the reference range applicable to healthy individuals with normally functioning adrenal glands. Currently, there is no detailed information on the influence of adrenalectomy on epinephrine and norepinephrine concentrations.

Therefore, the aim of this cohort study was to compare the urinary and plasma MN and NMN concentrations in patients who underwent unilateral or bilateral adrenalectomy for a pheochromocytoma with those determined in a healthy reference population.

Patients and Methods

Study population

We retrospectively studied patients above 20 years of age at the time of diagnosis who had undergone either bilateral or unilateral adrenalectomy for pheochromocytoma at the University Medical Center of Groningen between January 1980 and August 2011. This period was chosen because assays of fractionated total MNs in urine with gas chromatography-mass spectrometry and plasma fractionated free MNs with liquid chromatography tandem mass spectrometry (LC-MS/MS) have been available at our institution since 1979 and 2005, respectively. The diagnosis of a pheochromocytoma was histologically confirmed in all patients. Postoperative concentrations of MNs had to be available for at least 1 follow-up visit after adrenalectomy. Exclusion criteria were a recurrent tumor within 5 years of surgery, a partial (ie, cortical-sparing) adrenalectomy, and incomplete resection of the tumor. Patients who presented with a concurrent extra-adrenal paraganglioma were also excluded. Preferably, tumor recurrence had to be confirmed by histology. In cases lacking this information, a diagnosis of tumor recurrence was based on the combined results of anatomical and functional imaging.

The initial clinical diagnosis of a pheochromocytoma was based on elevated urinary and/or plasma MN and NMN concentrations and localization of the tumor with anatomical imaging of both adrenal glands (computed tomography/magnetic resonance imaging) as well as whole-body functional imaging

with [^{123}I]metaiodobenzylguanidine (MIBG) scintigraphy or [^{18}F]dihydroxyphenylalanine (DOPA) positron emission tomography (PET) (available at our institution since 2003). Long-term follow-up was performed by measurement of urinary and/or plasma MNs. Anatomical and/or functional imaging was repeated in patients with persistently elevated MNs to localize recurrent disease. In accordance with international guidelines, patients below 50 years of age were tested for germline mutations (16–19). In patients above 50 years of age, the family history was checked to evaluate the risk for hereditary syndromes.

For all patients, the following data were retrieved from their medical files: symptoms (headache, abdominal complaints, palpitations, paleness, flushes, vertigo, nausea, and anxiety), signs related to a pheochromocytoma (blood pressure [BP] and pulse rate) at presentation, pre- and postoperative use of medication, and results of germline mutation analysis. Hypertension was defined as a systolic BP of ≥140 mm Hg and/or a diastolic BP of ≥90 mm Hg or the use of antihypertensive medication. Additionally, we evaluated surgical and pathology reports of all the patients included in the study to confirm complete resection of the adrenal medulla. Because of the retrospective nature of this study and the use of clinical data, no further Institutional Review Board approval was required, according to the Dutch Medical Research Involving Human Subjects Act.

The concentrations of urinary and/or plasma MN and NMN were collected pre- and postoperatively, at 3 to 6 months and annually up to 5 years. The main outcome measure was the concentration of urinary or plasma MN and NMN concentration at 3 to 6 months after unilateral or bilateral adrenalectomy. Blood samples were collected by venipuncture, with patients in seated position, in 10-mL Vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey) containing K_2-EDTA solution as anticoagulant.

Reference values for deconjugated urinary MN and NMN concentrations were established in a group of 60 healthy subjects (30 men and 30 women, 20–70 years of age), as reported previously by Willemsen et al (20). Reference values for plasma free MN and NMN concentrations were determined in a group of 120 healthy subjects (63 men and 57 women, 36–81 years of age) participating in the Prevention of Renal and Vascular End Stage Disease (PREVEND) study (21, 22). Healthy subjects taking antihypertensive medication were not included. Measurement of plasma and urinary MN concentrations in both healthy subjects and patients was performed without prior dietary restrictions.

Analytical methods

Isotope-dilution mass spectrometry-based measurements of urinary and/or plasma MNs were used. Urinary deconjugated MN concentrations were determined by isotope-diluting gas chromatography-mass spectrometry, as described by Muskiet et al (23, 24). Urinary deconjugated MN concentrations were normalized to the urinary excretion of creatinine, measured using a picric acid-based method (before 2005) or an enzymatic method (Roche Diagnostics, Almere, The Netherlands) (after 2005) and expressed in units of micromoles per mole of creatinine. The intra-assay variation coefficient was 1.7%–4.2%, and the interassay variation coefficient was 3.3%–15.4%. Reference intervals for urinary MNs were as follows: MN, 33–99 μmol/mol creatinine; NMN, 64–260 μmol/mol creatinine.

Plasma free MN assays were performed with a HPLC tandem mass spectrometric technique (LC-MS/MS) with automated solid-phase extraction sample preparation, as described by de Jong et al (22). Established reference intervals for plasma free MNs were as follows: MN, 0.07–0.33 nmol/L; NMN, 0.23–1.07 nmol/L. The intra-assay and interassay variation coefficients were 2.5%–4.8% and 3.4%–5.6% for the free plasma MN measurement and 5.1%–6.2% and 4.2%–7.1% for the free plasma NMN measurement.

Statistics

Data are presented as mean ± SD or as median with interquartile ranges where appropriate. Differences between urinary MNs in healthy subjects and patients after unilateral or bilateral adrenalectomy were evaluated using the Mann-Whitney *U* test with Bonferroni correction. A Mann-Whitney *U* test was performed to calculate the differences between the reference population and patients after unilateral adrenalectomy for the plasma MNs. Baseline was defined as 3 to 6 months after adrenalectomy. For each individual, a Spearman correlation coefficient was calculated regarding the relationship of the respective plasma and urinary MNs concentrations with follow-up time (ie, baseline and the various follow-up time points). Subsequently, averaged correlation coefficients were calculated after Fisher's Z-transformation using the correlation coefficients per patient. We also calculated the percent changes in plasma and urinary MNs at the various follow-up moments compared with baseline. A two sided P value $<.05$ was considered statistically significant. Statistical analyses were performed with PASW statistics (version 18.0; IBM/SPSS, Armonk, New York).

Results

Patient characteristics

Between January 1980 and August 2011, 108 pheochromocytoma patients underwent a unilateral or bilateral adrenalectomy at our institution. Of these, 14 patients (13.0%) were excluded. Reasons for exclusion were missing laboratory data (n = 4), subtotal adrenalectomy (n = 1), and recurrent disease (n = 9) within 5 years after adrenalectomy. Table 1 shows the preoperative characteristics of all patients included in the study. In 2 patients (2.1%) with a sporadic pheochromocytoma, no functional imaging was performed.

Seventy patients (74.5%) underwent a unilateral adrenalectomy. Median follow-up time for these patients was 39.2 (14.9–86.2) months. During follow-up, 4 patients (5.7%) died (cardiovascular disease, n = 1; malignancy, n = 2; unknown cause, n = 1) after a median follow-up time of 17.0 (3.9–30.5) months. Twelve patients (17.1%) were referred to other centers for continuation of follow-up after a median 19.6 (8.4–49.0) months.

Twenty-four patients (25.5%) underwent a bilateral adrenalectomy. The median follow-up time was 169.5 (56.8–235.4) months. During follow-up, 4 patients (16.7%) died (malignancy, n = 1; gastrointestinal bleeding, n = 1; suicide, n = 1; unknown cause, n = 1) after a median period of 42.6 (3.3–132.1) months, and in 1 patient (4.2%), follow-up was continued at another center after 128.4 months.

Preoperatively, all patients were treated with adrenergic receptor antagonists to prevent potentially fatal complica-

Table 1. Patient Characteristics Before Adrenalectomy

	Patient Data (n = 94)
Sex (male/female)	38/56
Age, y (mean ± SD)	47 ± 16
Adrenalectomy, n (%)	
Unilateral, left	33 (35)
Unilateral, right	37 (39)
Bilateral	24 (26)
Functional Imaging, n (%)	
Whole-body [^{123}I]MIBG	75 (80)
Whole-body [^{18}F]DOPA PET	48 (51)
Pathology, median (IQR)	
Adrenal volume, cm^3	54 (30–224)
Weight, g	38.8 (21.2–121.5)
Germline mutations, n (%)	
None (sporadic)	60 (64)
Familiar syndrome	34 (36)
MEN 2a	22 (65)
MEN 2b	2 (6)
VHL	5 (15)
Neurofibromatosis type 1	3 (9)
SDHB	1 (3)
SDHD	1 (3)
Main reason for referral, n (%)	
Tumor on anatomical imaging	22 (23)
Analysis hypertension	23 (24)
Symptoms related to pheochromocytoma	25 (27)
Known mutation carrier	23 (24)
Unknown	1 (1)
Hemodynamic control	
Mean BP ± SD, mm Hg	139 ± 31/85 ±17
Mean pulse ± SD, beats/min	81 ± 13
Hypertension, n %	66 (70)
Antihypertensive medication, n (%)	39 (43)
β-Adrenergic receptor antagonists	25 (26)
α-Adrenergic receptor antagonists	14 (15)
Angiotensin-II-receptor antagonists	5 (5)
Calcium channel blockers	4 (4)
ACE inhibitors	9 (9)
Diuretics	11 (12)
Preoperative biochemistry, median (IQR)[a]	
Urinary MN, μmol/mol creatinine	898 (178–3286)
Urinary NMN, μmol/mol creatinine	1160 (333–2499)
Plasma MN, nmol/L	1.03 (0.28–4.35)
Plasma NMN, nmol/L	4.31 (1.26–13.36)

Abbreviations: ACE, angiotensin-converting enzyme; SDH, succinate dehydrogenase; VHL, von Hippel-Lindau; IQR, interquartile range.

[a] Reference ranges for urinary metanephrines were as follows: MN, 33–99 μmol/mol creatinine; NMN, 64–260 μmol/mol creatinine. Reference ranges for plasma metanephrines were as follows: MN, 0.07–0.33 nmol/L; NMN, 0.23–1.07 nmol/L.

tions of surgery. At 3 to 6 months after adrenalectomy, 24 patients (25.5%) used antihypertensive medication, of whom 13 used β-adrenergic receptor antagonists (13.8%).

Postoperative deconjugated urinary and plasma free MN concentrations

In patients who underwent a unilateral adrenalectomy, the median urinary and plasma MN concentration were significantly lower 3 to 6 months after unilateral adrenalectomy compared with the reference population (both $P < .05$), as shown in Table 2. The median urinary MN concentration 3 to 6 months after unilateral adrenalectomy was 36.1% (13.1%–49.2%) lower, whereas the median plasma MN concentration was 22.3% (0.0%–50.0%) lower compared with the median values in the reference population. In 19 patients (33.9%), the urinary MN concentration was below the lower reference limit (LRL) 3 to 6 months after unilateral adrenalectomy. The plasma MN concentration was below the LRL in 2 patients (6.7%) 3 to 6 months after adrenalectomy. During the 5-year follow-up, there was a median increase of the urinary MN concentration of 11.1% (−8.5%–36.9%) compared with baseline values (defined as 3 to 6 months after adrenalectomy) (averaged correlation coefficient, 0.37; $P < .05$). This correlates to a small absolute increase of the median urinary MN concentration from 39 to 43 μmol/mol creatinine. There were no changes over the 5-year time frame in plasma MN (averaged correlation coefficient, 0.19; $P = .058$) (Figure 2). In patients who underwent a bilateral adrenalectomy, the median urinary MN concentration 3 to 6 months after adrenalectomy was 88.5% (63.9%–98.4%) lower compared with the reference population ($P < .05$) (Table 2). Fifteen patients (83.3%) had a urinary MN concentration below the LRL 3 to 6 months after bilateral adrenalectomy. There was a median decrease of 55.3% (−77.0%–44.6%) in urinary MN after bilateral adrenalectomy during the 5 years of follow-up (averaged correlation coefficient, −0.28; $P < .05$) (Figure 3). The plasma MN concentration was available for only 2 patients and was 0.01 and 0.03 nmol/L at 3 to 6 months, respectively; therefore, no statistical comparison was made (Table 2). The urinary MN concentration of 17 patients with MEN type 2A 3 to 6 months after bilateral adrenalectomy was 4 (1–11.8) μmol/mol creatinine, which was lower compared with the reference population ($P < .05$).

Postoperative deconjugated urinary and plasma free NMN concentrations

Median urinary and plasma NMN concentrations in patients after unilateral adrenalectomy were higher compared with the reference population 3 to 6 months after unilateral adrenalectomy (both $P < .05$) (Table 2). The median urinary NMN concentration 3 to 6 months after unilateral adrenalectomy was 32.5% (2.6%–65.8%) higher compared with the median value in the reference population, whereas the median plasma NMN concentration was 47.2% (11.3%–88.7%) higher. The urinary NMN concentration 3 to 6 months after unilateral adrenalectomy was above the upper reference limit (URL) in 6 patients (10.7%), with concentrations ranging from 268 to 537 μmol/mol creatinine. Four

Table 2. Plasma and Urinary MNs in Patients 3 to 6 Months After Unilateral and Bilateral Adrenalectomy[a]

	Unilateral Adrenalectomy	Bilateral Adrenalectomy	Reference Population
Urinary MN, μmol/mol creatinine	39 (31–53)[b]	7 (1–22)[b]	61 (49–74)
n	58	18	60
Plasma MN, nmol/L	0.14 (0.09–0.18)[c]	0.01 and 0.03	0.18 (0.13–0.23)
n	30	2	120
Urinary NMN, μmol/mol creatinine	151 (117–189)[b]	177 (106–238)[b]	114 (98–176)
n	58	18	60
Plasma NMN, nmol/L	0.78 (0.59–1.00)[c]	0.52 and 0.67	0.53 (0.41–0.70)
n	30	2	120

[a] Data are shown as median (interquartile range).

[b] $P < .05$, both calculated for urinary MNs between the reference population and patients after unilateral or bilateral adrenalectomy and with Bonferroni correction.

[c] $P < .05$, calculated for plasma MNs between the reference population and patients after unilateral adrenalectomy. Because of the low number of patients, no comparison was made between plasma MNs after bilateral adrenalectomy and the reference population.

patients (13.3%) had a plasma NMN concentration above the URL 3 to 6 months after unilateral adrenalectomy. In the 5 years of follow-up, there was a median increase in urinary NMN concentration of 11.2% (−21.6%–22.4%) compared with the baseline values (averaged correlation coefficient, 0.14; $P < .05$). There was no change in plasma NMN after unilateral adrenalectomy over the time frame of 5 years (averaged correlation coefficient, −0.03; $P = .788$) (Figure 2).

In patients who underwent a bilateral adrenalectomy, the median urinary NMN concentration significantly increased compared with the reference population ($P < .05$). Three patients (16.7%) had a urinary NMN concentration above the URL 3 to 6 months after bilateral adrenalectomy with concentrations ranging from 382 to 430 μmol/mol creatinine. There were no changes during the 5 years of follow-up in the urinary NMN after bilateral adrenalectomy (averaged correlation coefficient, −0.00; $P =$

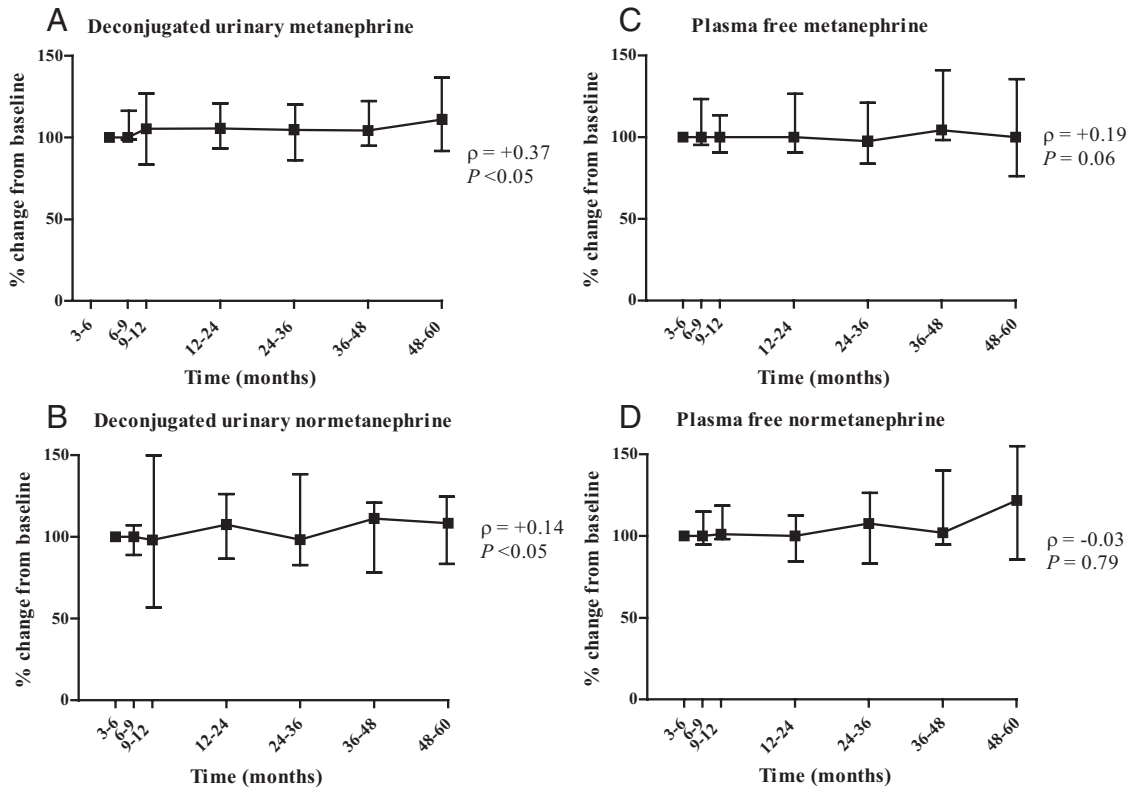

Figure 2. Percent changes from baseline (3 to 6 months after adrenalectomy) of urinary and plasma MNs in patients after unilateral adrenalectomy. Baseline measurements are set at 100%. Graphs depicting the percent changes with interquartile ranges of urinary and plasma MNs from baseline. In deconjugated urinary MN (A) and deconjugated urinary NMN (B), there was a change of 11.1% and 11.2% during 5-year follow-up compared with baseline values (3 to 6 months after adrenalectomy). In plasma free MN (C) and plasma free NMN (D), there is no significant change in a 5-year time frame.

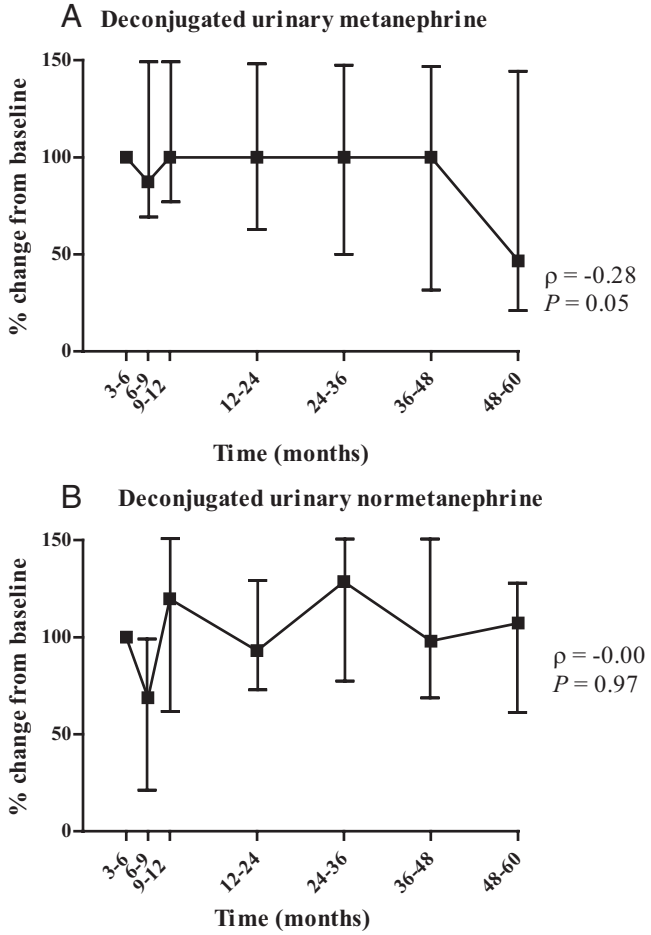

Figure 3. Percent changes from baseline (3 to 6 months after adrenalectomy) of urinary MNs in patients after bilateral adrenalectomy. Baseline measurements are set at 100%. Graphs depicting the percent change with interquartile ranges of urinary MNs after bilateral adrenalectomy from baseline. Deconjugated urinary MN (A) and deconjugated urinary NMN (B) show no significant change of urinary MN and urinary NMN in a 5-year time frame. Plasma MN and NMN concentrations after bilateral adrenalectomy are not shown because of the low number of patients in this group.

.972) (Figure 3). The plasma NMN concentration was 0.52 and 0.67 nmol/L 3 to 6 months after bilateral adrenalectomy in the 2 patients in whom this was available. The urinary NMN concentration of 17 patients with MEN type 2A 3 to 6 months after bilateral adrenalectomy was higher compared with the reference population, 177 (102–232) μmol/mol creatinine ($P = .09$).

In 17 patients (18.1%), an increased urinary and/or plasma NMN concentration was found during one or more follow-up visits in the 5-year follow-up period. In 7 of these 17 patients, additional imaging with computed tomography (n = 2), magnetic resonance imaging (n = 2), [^{123}I]MIBG (n = 5), [^{18}F]DOPA PET (n = 2), [^{111}In] Octreoscan (n = 1), or a clonidine suppression test (n = 1) was performed with no evidence of recurrent disease. In all of these 17 patients, the NMN concentration normalized during follow-up without intervention.

Discussion

In the present study, we found that MN concentrations in both plasma and urine after unilateral and bilateral adrenalectomy were persistently lower compared with the values obtained in a reference population consisting of healthy subjects. As expected, this difference was most profound in patients after bilateral adrenalectomy. In contrast, we found that NMN concentrations in both plasma and urine in patients after adrenalectomy were higher than in healthy subjects.

Two previous studies have described the catecholamine and MN concentration after bilateral adrenalectomy. Shah et al (25) demonstrated that plasma epinephrine and norepinephrine were still detectable after bilateral adrenalectomy in a small group of 5 patients with Cushing's syndrome. Eisenhofer et al (26) studied 12 patients after bilateral adrenalectomy, of whom 11 patients had Cushing's syndrome and 1 patient had a pheochromocytoma. In concordance with our study, they found that the plasma epinephrine concentration was below the limit of detection, and plasma concentrations of free and conjugated MN were decreased compared with healthy control subjects. Plasma NMN concentrations, however, were unaffected in that study (26). It should be noted that the present study is considerably larger, includes patients after unilateral adrenalectomy, and contains long-term follow-up data. Furthermore, the liquid chromatography with electrochemical detection used in the latter study is more susceptible to interference and has a higher detection limit and is therefore not as accurate as the LC-MS/MS technique we applied (27).

Although we observed a small statistically significant increase of 4 μmol/mol creatinine in the urinary MN concentration during the 5-year follow-up, the urinary MN concentration is persistently lower after unilateral adrenalectomy. This implies that the contralateral adrenal gland cannot fully compensate for the loss of production and secretion of epinephrine. The detection of MN in patients after bilateral adrenalectomy suggests that epinephrine production might take place outside the adrenal gland. This is supported by the demonstration of positive immunohistochemical staining for PNMT in adrenergic cells present in fetal rat hearts and in rat adipocytes (28–30).

There might be several explanations for the observed increase in postsurgical NMN concentrations. Most likely, this increase is explained by an augmented production of norepinephrine in sympathetic nerves, thereby compensating for the loss of epinephrine activity in maintaining vascular tone. Alternatively, this phenomenon could also result from desensitization of α- and β-receptors due to the high amount of circulating catecholamines before adrenalectomy, but this phenomenon is temporary

and therefore expected to resolve after adrenalectomy (31). Because patients with tumor recurrence within 5 years after surgery were excluded, the increase cannot be attributed to a recurrent pheochromocytoma. This is additionally supported by the observation that the increase in NMN concentrations had already occurred 3 to 6 months postoperatively and the increase thereafter was only marginal. False-positive results caused by analytical interference are not likely because we used mass spectrometric detection, which is known for its very high analytical specificity (22). It could be argued that our results might be confounded by preanalytical factors such as pharmacological interference by antihypertensive drugs, the lack of dietary restrictions, or patient positioning during blood sampling. However, analyzing patients without antihypertensive medication did not change the results (data not shown). In the present study, blood was collected while healthy subjects and patients were seated. It has been demonstrated that plasma MNs are moderately elevated in the seated compared with the supine position (32). Our conclusions, however, will not be affected by the position during sampling, because the postoperative concentrations of plasma were compared with the reference population in whom blood sampling had also been performed in the seated position. In addition, the concomitant increase in 24-hour urinary NMN concentrations argues against a possible influence of body position during blood collection. Dietary restrictions of catecholamine-rich food supplies are considered to minimize the possibility of false-positive results for urinary deconjugated NMN concentrations. We found decreased concentrations of both urinary deconjugated and plasma free NMN concentrations. Therefore, it seems unlikely that diet is a confounding factor (21).

Our results strongly suggest that the diagnostic yield of the measurement of MNs in plasma or urine is negatively affected by adrenalectomy. The decrease in MN levels is expected to reduce the sensitivity, whereas the increase in NMN levels after adrenalectomy is likely to lower the specificity of this assay. Consequently, adjusted reference intervals are needed for patients with pheochromocytoma after unilateral or bilateral adrenalectomy. Downward adjustment of the MN reference ranges in these patients could thus contribute to an earlier detection of tumor recurrence, whereas upward adjustment of the NMN reference ranges could prevent unnecessary diagnostic tests. The latter might be particularly relevant for patients with von Hippel-Lindau syndrome, which is accompanied by an increased secretion of NMN (33). According to current knowledge, additional imaging could have been prevented during follow-up in 7 of 94 patients (7.5%) seen in our hospital after adrenalectomy because of a pheochromocytoma. Because imaging was not performed in patients with MN concentrations within the reference intervals, we cannot give an indication about how much sooner tumor recurrence would have been detected with adjusted reference intervals for MN. Specific reference intervals for urine and/or plasma MN and NMN need to be established in patients after adrenalectomy to improve both sensitivity and specificity for this patient group. Partial adrenalectomy is an emerging alternative procedure reducing the risk of adrenal insufficiency when performed by an experienced surgeon. Because the adrenal medulla is completely resected in partial adrenalectomy, the effects on the catecholamine secretion after successful resection of the pheochromocytoma are comparable with patients after complete adrenalectomy (34, 35). We, therefore, believe that our results are also applicable in patients after partial adrenalectomy.

Our study has some limitations. Because of the retrospective nature of this study, not all data are complete. Plasma MN concentrations after bilateral adrenalectomy were available in only a few patients, because this particular assay was introduced in our laboratory only after 2005. Patients with tumor recurrence after adrenalectomy within the observation period were excluded, but follow-up was shorter than 5 years in a minority of patients. In particular, in patients with hereditary tumor syndromes, tumor recurrence could not always be completely excluded even in the absence of detectable tumor on imaging. Furthermore, the number of patients was too small to perform statistical tests on the different hereditary groups. However, analyzing patients with MEN type 2a after bilateral adrenalectomy gave comparable results.

From these data, it can be concluded that adrenalectomy in pheochromocytoma patients results in a new set point in catecholamine production, with lower epinephrine and higher norepinephrine secretion. We therefore recommend the use of adapted reference intervals in the follow-up of pheochromocytoma patients after adrenalectomy. These reference intervals need to be established prospectively in a large population of patients who have undergone surgery for a sporadic or hereditary pheochromocytoma. These adapted reference intervals for MNs would be expected to enable both earlier detection of recurrent disease and prevent unnecessary diagnostic testing.

Acknowledgments

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