Dietary Influences on Plasma and Urinary Metanephrines: Implications for Diagnosis of Catecholamine-Producing Tumors

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Context: Measurements of the 3-O-methylated metabolites of catecholamines [metanephrines (MNs)] in plasma or urine are recommended for diagnosis of pheochromocytoma. It is unclear whether these tests are susceptible to dietary influences.

Objective: The aim of the study was to determine the short-term influence of a catecholamine-rich diet on plasma and urinary fractionated MNs.

Design, Setting, and Participants: We conducted a crossover study in a specialist medical center involving 26 healthy adults.

Interventions: Subjects consumed catecholamine-rich nuts and fruits at fixed times on one day (about 35 μmol dopamine and 1 μmol norepinephrine) and catecholamine-poor products on another day. Blood and urine samples were collected at timed intervals before, during, and after experimental and control interventions.

Main Outcome Measures: Isotope-dilution mass spectrometry-based measurements of plasma and urinary concentrations of free and deconjugated 3-methoxytyramine (3-MT), normetanephrine (NMN), and MN were made.

Results: The catecholamine-rich diet had substantial effects (up to 3-fold increases) on plasma concentrations and urinary outputs of free and deconjugated 3-MT. Dietary catecholamines had negligible influences on free NMN in plasma and urine, but substantial effects (up to 2-fold increases) on deconjugated NMN in plasma and urine. Concentrations of free and deconjugated MN in plasma and urine remained unaffected.

Conclusions: Dietary restrictions should be considered to minimize false-positive results for urinary and plasma deconjugated MNs during diagnosis of pheochromocytoma. Similar considerations appear warranted for plasma and urinary free 3-MT, but not for free NMN or MN, indicating advantages of measurements of the free compared to deconjugated metabolites. **(*J Clin Endocrinol Metab* 94: 2841–2849, 2009)**

The diagnosis of pheochromocytoma depends on demonstration of elevated production of catecholamines, usually achieved by analysis of plasma and urinary free catecholamines and catecholamine metabolites. The 3-O-methylated metabolites (fractionated metanephrines), including metanephrine (MN) produced from epinephrine; normetanephrine (NMN) from norepinephrine; and 3-methoxytyramine (3-MT) from dopamine; are particularly useful (1–3). The MNs are produced within tumor cells where the presence of membrane-bound catechol-O-methyltransferase leads to metabolism of catecholamines leaking from storage vesicles into

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Abbreviations: MN, Metanephrine; 3-MT, 3-methoxytyramine; NMN, normetanephrine.

the cytoplasm (4). This process is continuous and independent of variations in catecholamine release, providing diagnostic advantages for MNs compared with the parent amines. Measurements of MNs in plasma or urine are therefore currently recommended for the diagnosis of pheochromocytoma (5–9), with some groups advocating use of the plasma test (5, 6) and others the urinary test in combination with measurements of catecholamines (8, 9).

We recently developed an automated online extraction-HPLC-tandem mass spectrometric method with high analytical performance for measurement of plasma free MNs (10). However, biochemical results may be affected by preanalytical factors, such as physiological influences (*e.g.* exercise, posture, stress) and medications (*e.g.* catecholamine reuptake blockers) that alter the production or disposition of catecholamines and their metabolites. Dietary influences represent other factors that may affect biochemical tests of catecholamine excess. In particular, numerous food products, such as fruits and nuts, contain substantial quantities of biogenic amines that may produce false-positive test results (11–13).

Catecholamines and MNs are converted to sulfate conjugates (Fig. 1) by a sulfotransferase isoenzyme, SULT1A3, located in the gastrointestinal tract, that inactivates both endogenous and dietary-derived (exogenous) catecholamines (14–16). Consequently, consumption of catecholamine-containing foods can lead to large increases in sulfate-conjugated catecholamines, particularly dopamine sulfate (15–17). Because MNs in urine are commonly measured after an acid-hydrolysis deconjugation step and largely reflect sulfate-conjugated metabolites, an influence of food-derived catecholamines may be important to consider for those measurements. Although the influences of dietary catecholamines on biochemical test results have been studied previously (13, 17–19), there are no clear data concerning these influences on concentrations of free or deconjugated MNs.

The primary aim of this study was to determine the influence of a catecholamine-rich diet on free and deconjugated MNs. Plasma and urinary free and deconjugated MNs were measured to determine the relative influences of dietary constituents on each of the four tests. We also examined the influence of blood sampling in seated *vs.* supine positions to assess previous recommendations that samples should be collected after supine rest (6, 20).

Subjects and Methods

Subjects and diet experiment

Subjects included 26 healthy adults (13 women, 13 men; median age, 38 yr; range, 21–59 yr) who served as their own controls by participating in both control and experimental arms of the protocol following a cross-

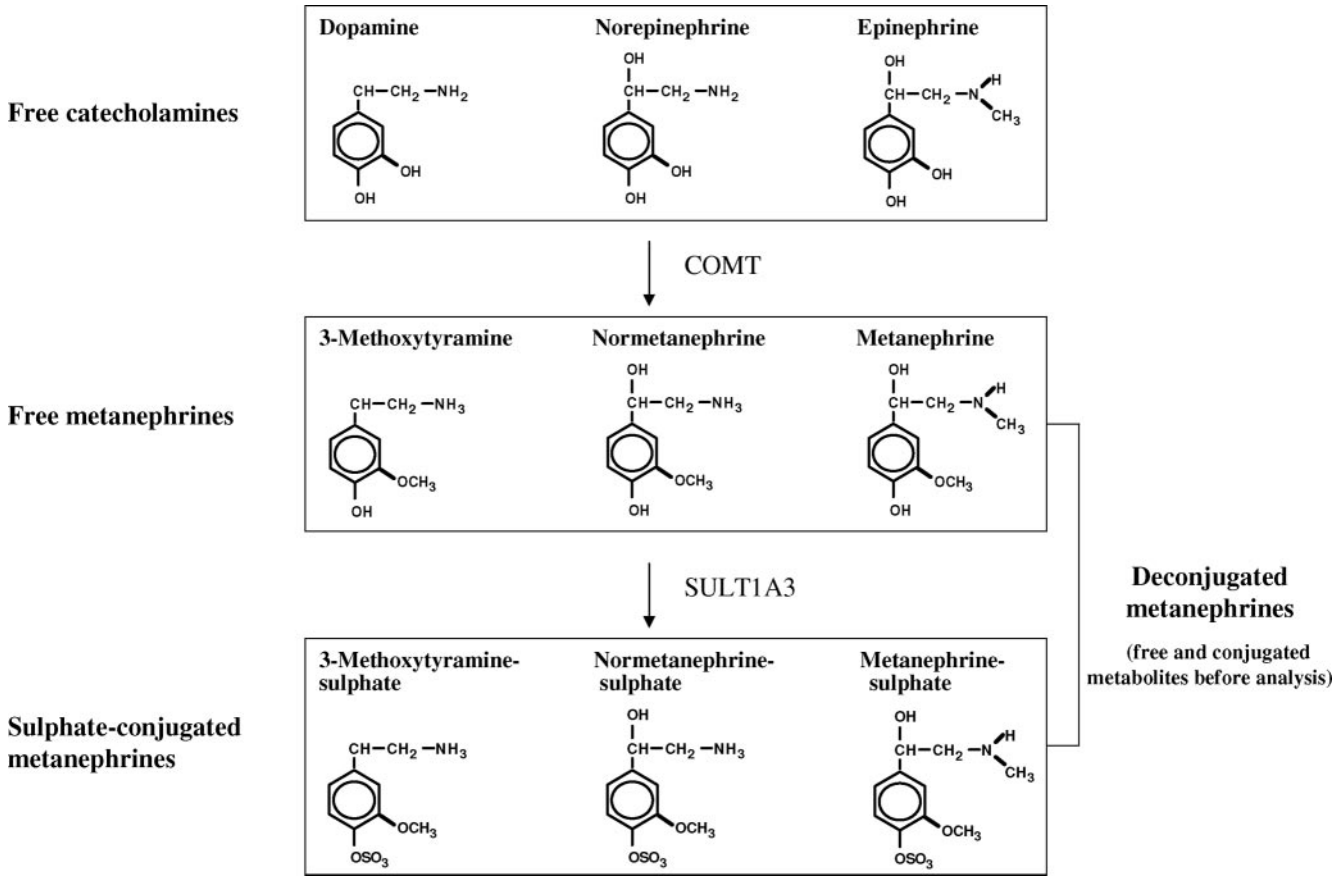

FIG. 1. Simplified diagram showing the metabolism of catecholamines to free and sulfate-conjugated MNs before analysis. Dopamine, norepinephrine, and epinephrine are metabolized to free 3-MT, NMN, and MN, respectively, by the enzyme catechol-O-methyltransferase (COMT). The free MNs may then be conjugated with a sulfate group by the sulfotransferase isoenzyme 1A3 (SULT1A3). MNs may be measured in plasma or urine in either the free form or after a deconjugation step, the latter commonly employed for measurements of the urinary metabolites. The deconjugated MNs mainly represent sulfate-conjugated metabolites. This study included all four measurements (*i.e.* plasma free MNs, plasma deconjugated MNs, urinary free MNs, and urine deconjugated MNs).

over design, with at least 1 wk between the randomly distributed test days. Both arms were preceded by an overnight fast from at least 2400 h until 0830 h. Subjects avoided catecholamine-containing products (*e.g.* fruits, fruit drinks, nuts, potatoes, tomatoes, and beans) the day before, during, and the morning after both study days. The study design was based on a previous diet intervention study (13).

For the experimental arm, all subjects consumed two catecholamine-rich meals. The first meal, consumed at 0830 h, included two or three bananas (280 g of pulp), one fourth of a fresh pineapple (185 g of pulp), 50 g shelled walnuts, and 140 ml pineapple juice, purchased at local commercial outlets. At 1030 h each subject drank 280 ml pineapple juice. At 1230 h they consumed a second meal similar to the first. Finally, at 1430 h, participants drank 280 ml pineapple juice. Subjects avoided catecholamine-rich dietary products until the next morning. Based on previous measurements (13), total dopamine and norepinephrine intakes were estimated at 35 and 1 μmol, respectively; epinephrine was undetectable.

For the control arm, the subjects consumed meals (bread), snacks (gingerbread), and drinks (coffee, tea, dairy products) in accordance with the time schedule of the experimental arm. The study was approved by the medical ethics committee of our institution and conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent.

Sample collection

Morning urine until 0830 h was collected from all subjects. Four separate urine samples were then collected at 2-h intervals starting at 1030 h, with the fourth collection at 1630 h. A final urine specimen was collected beginning at 1630 h and ending at 0800 h the next morning (see Table 2). All urine samples were collected without acidification or preservative. Aliquots were stored within 1 d at −20 C until analysis within 6 months after collection.

Blood samples (10 ml) were collected into EDTA-containing Vacutainer tubes (Becton and Dickinson, Franklin Lakes, NJ) using an indwelling Braunule catheter (Braun, Melsungen, Germany) inserted into a forearm vein at 0800 h. The first blood sample was drawn immediately after insertion of the catheter with subjects in the seated position. A second blood sample was drawn after 30 min of supine rest (0830 h), immediately before the first meal. All subsequent blood samples were drawn after resting for 30 min in the supine position. These samples were collected at 1030, 1230, and 1430 h before test meals or drinks, and finally at 1630 h. All blood samples were immediately centrifuged at $2{,}500 \times g$ for 10 min at 4 C. Plasma samples were stored at −20 C until analyses within 3 months after collection. The procedures for urine and blood sampling were carried out identically on both control and experimental days.

Analytical methods

Plasma free and deconjugated MNs and urinary free MNs were measured using an online extraction-HPLC-tandem mass spectrometric method (10). Plasma deconjugated MNs were determined after acid hydrolysis, carried out by incubating 1 ml of water-diluted (1:1) plasma with 15 μl of perchloric acid (pH 1.0) over 20 min at 100 C (21). Urinary deconjugated MNs were determined by isotope-dilution gas chromatography-mass spectrometry, as previously described (22). Urinary outputs of free and deconjugated MNs were normalized to the urinary excretion of creatinine, measured using an enzymatic method (Roche Diagnostics, Almere, The Netherlands), and expressed in units of micromoles per mole creatinine.

Reference intervals

Reference intervals for plasma deconjugated MNs were determined using blood samples collected in the seated position from 115 volunteers (57 males, 58 females; age range, 36–81 yr; median age, 55 yr) who participated in the PREVEND study (23). Reference intervals for urinary free MNs were obtained from the analysis of 120 24-h urine samples that were collected from healthy subjects participating in the LifeLines study (24). Reference values for plasma free and urinary deconjugated MNs have been reported elsewhere (10, 22). All reference intervals were determined without preceding dietary restrictions and calculated using EP Evaluator software (DG Rhoads Associates, Inc., Kennett Square, PA) as recommended by the Clinical and Laboratory Standards Institute.

Data analysis and statistics

Data are shown as mean values with 95% confidence intervals. The influence of blood sampling in seated *vs.* supine positions was tested using paired *t* test (SPSS version 16; SPSS Inc., Chicago, IL). The plasma samples collected at 0830 h and the first morning urine samples (until 0830 h) served as reference points for dietary-associated changes over time. Linear mixed models, tested for significance at $P < 0.05$, were used to determine the significance of temporal changes in analyte concentrations (25). The model fit was evaluated on deviance and performed using the statistic software program ML Win version 2.0.2 (Centre for Multilevel Modeling, Bristol, UK). Changes over time were modeled in agreement with the findings of descriptive statistics (SPSS). The magnitude of a difference between the control and the experimental groups in this modeling is given by the interaction term between diet and time because no differences at baseline concentrations between both groups were expected. Time was included as a covariate when possible, or otherwise as a factor.

Results

Plasma concentrations of free and deconjugated MNs exhibited divergent responses to dietary manipulations and the different postural conditions of blood sampling (Table 1).

Influence of sampling position

Plasma free NMN and MN were 30 and 12% higher, respectively ($P < 0.001$), in the blood sample collected in the seated position (0800 h sample) than that collected in the supine position (0830 h sample) after a 30-min rest (Fig. 2). In contrast, there were no influences of these sampling conditions on plasma free 3-MT or deconjugated NMN, MN, or 3-MT.

Influence of diet on plasma 3-MT

Plasma free 3-MT and deconjugated 3-MT differed significantly ($P < 0.05$) over the course of the day of the high-catecholamine diet compared with the control diet (Fig. 3, A and B). Plasma concentrations of both dopamine metabolites increased ($P < 0.05$) after the first catecholamine-rich meal, followed by stabilization until the next meal. After the second catecholamine-rich meal, plasma deconjugated 3-MT increased more than 3-fold compared with baseline levels ($P < 0.05$), whereas plasma free 3-MT increased 2-fold (Table 1 and Fig. 3, A and B). Both increases were followed by smaller but significant decreases. Plasma free 3-MT (Fig. 3A) showed no consistent change during the day of the control diet. In contrast, plasma deconjugated 3-MT (Fig. 3B) followed the same pattern observed on the day of the catecholamine-rich meals, albeit with much smaller but still significant increases after the consumption of each meal.

Influence of diet on plasma NMN

In contrast to the results for plasma 3-MT (Fig. 3A), plasma free NMN showed no significant differences during the days that subjects consumed the catecholamine-rich meals compared with

TABLE 1. Plasma concentrations of MNs before, during, and after ingestion of catecholamine-rich and catecholamine-poor food products

Control	Time and position during blood sampling						↑ Reference limit
	0800 h seated	0830 h supine[a]	1030 h supine	1230 h supine	1430 h supine	1630 h supine	
3-MT free							0.17
Control	0.08 (0.08–0.09)	0.08 (0.07–0.08)	0.08 (0.07–0.09)	0.09 (0.08–0.09)	0.09 (0.08–0.09)	0.10 (0.08–0.11)	
Diet	0.08 (0.08–0.09)	0.09 (0.07–0.10)	0.15 (0.13–0.16)	0.15 (0.13–0.16)	0.18 (0.15–0.20)[b]	0.14 (0.13–0.16)	
3-MT deconjugated							19.12
Control	4.57 (4.00–5.14)	4.46 (3.93–4.99)	5.80 (5.15–6.46)	5.74 (5.12–6.37)	6.68 (5.87–7.48)	6.07 (5.36–6.78)	
Diet	5.44 (4.79–6.08)	5.35 (4.76–5.93)	12.08 (11.12–13.03)	13.28 (12.08–14.48)	18.49 (16.56–20.41)	16.76 (14.63–18.89)	
NMN free							1.14
Control	0.54 (0.46–0.61)	0.41 (0.36–0.47)	0.43 (0.37–0.49)	0.47 (0.42–0.52)	0.43 (0.38–0.49)	0.46 (0.40–0.52)	
Diet	0.57 (0.50–0.63)	0.44 (0.38–0.49)	0.48 (0.41–0.56)	0.53 (0.46–0.59)	0.50 (0.43–0.57)	0.50 (0.44–0.57)	
NMN deconjugated							39.03
Control	9.38 (7.96–10.79)	9.24 (7.95–10.53)	9.72 (8.33–11.12)	10.17 (8.87–11.48)	10.35 (9.04–11.67)	10.29 (9.01–11.57)	
Diet	10.19 (9.18–11.20)	10.13 (9.12–11.14)	15.00 (13.53–16.47)	14.22 (12.84–15.59)	18.35 (16.07–20.63)	16.50 (14.51–18.50)	
MN free							0.34
Control	0.23 (0.20–0.26)	0.21 (0.18–0.24)	0.18 (0.16–0.21)	0.20 (0.18–0.23)	0.19 (0.16–0.22)	0.22 (0.18–0.25)	
Diet	0.24 (0.21–0.28)	0.21 (0.18–0.25)	0.20 (0.16–0.23)	0.21 (0.18–0.24)	0.20 (0.17–0.23)	0.21 (0.17–0.24)	
MN deconjugated							12.26
Control	4.60 (3.78–5.41)	4.54 (3.80–5.29)	4.77 (3.93–5.61)	4.73 (3.96–5.49)	4.76 (4.04–5.47)	4.69 (4.00–5.39)	
Diet	5.05 (4.26–5.83)	5.09 (4.29–5.88)	5.47 (4.65–6.30)	5.31 (4.50–6.11)	5.52 (4.63–6.42)	5.42 (4.54–6.31)	

Test meals were taken at 0830 and 1230 h; test drinks at 1030 and 1430 h. Mean plasma concentrations are given in nanomoles per liter (n = 26). Data within *parentheses* denote 95% confidence intervals. Deconjugated fractions refer to free + conjugated metanephrines. Because of the used model fit statistics, significant changes over time are not expressed in this table. Note: Upper limits of reference intervals (Reference limits) were determined from samples collected in the seated position and without dietary restrictions.

[a] Baseline time.

[b] Above reference value.

the control meals (Fig. 3C). Concentrations in both groups gradually increased during the day, starting after the first supine baseline sample at 0830 h. In contrast to free NMN (Fig. 3C), but similar to plasma deconjugated 3-MT (Fig. 3B), plasma deconjugated NMN differed significantly ($P < 0.05$) after high-catecholamine meals compared with control meals (Fig. 3D and Table 1). More specifically, plasma deconjugated NMN showed successive increases ($P < 0.05$) after each of the two meals, with smaller but significant intervening decreases thereafter. After the second meal, levels of deconjugated NMN increased nearly 2-fold compared with baseline values. Plasma deconjugated NMN also increased slightly after control meals, but the increase only reached significance after the second meal.

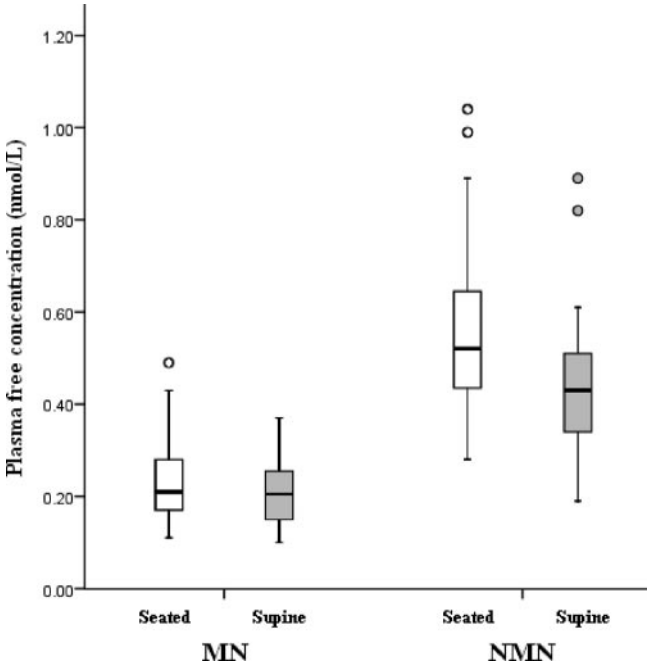

FIG. 2. Differences in plasma concentrations of free MN and NMN after blood sampling in the seated position compared with after 30 min of supine rest. Data from 26 subjects are shown graphically. Decreases in mean MN and NMN concentration after 30 min of supine rest are significant ($P < 0.001$). The *single dots* are outliers.

Influence of diet on plasma MN

Similar to plasma free NMN (Fig. 3C), plasma free MN did not differ between the days that subjects ingested catecholamine-rich and control meals (Fig. 3E). Levels remained constant over the course of both days. For plasma deconjugated MN (Table 1 and Fig. 3F), there were large interindividual variations that made detection of between-group differences difficult. However, following the model fit formula, the significant increase ($P < 0.05$) of plasma deconjugated MN in the diet group appeared similar to that of the control group (Table 1), which implies no influence of the catecholamine-rich diet on plasma deconjugated MN.

Influence of diet on urinary 3-MT

Urinary outputs of free and deconjugated 3-MT were significantly ($P < 0.05$) higher after ingestion of the high-catecholamine meals compared with control meals. After consumption of the second catecholamine-rich meal, urinary outputs of free 3-MT (Table 2 and Fig. 4A) were nearly 2-fold ($P < 0.05$) and deconjugated 3-MT more than 2-fold higher ($P < 0.05$) than baseline values (Table 2 and Fig. 4B). Urinary free 3-MT returned to baseline values overnight, whereas urinary deconjugated 3-MT remained elevated, but decreased significantly from values of the preceding collection. No significant changes in urinary concentrations of free or deconjugated 3-MT were apparent after ingestion of the control meal.

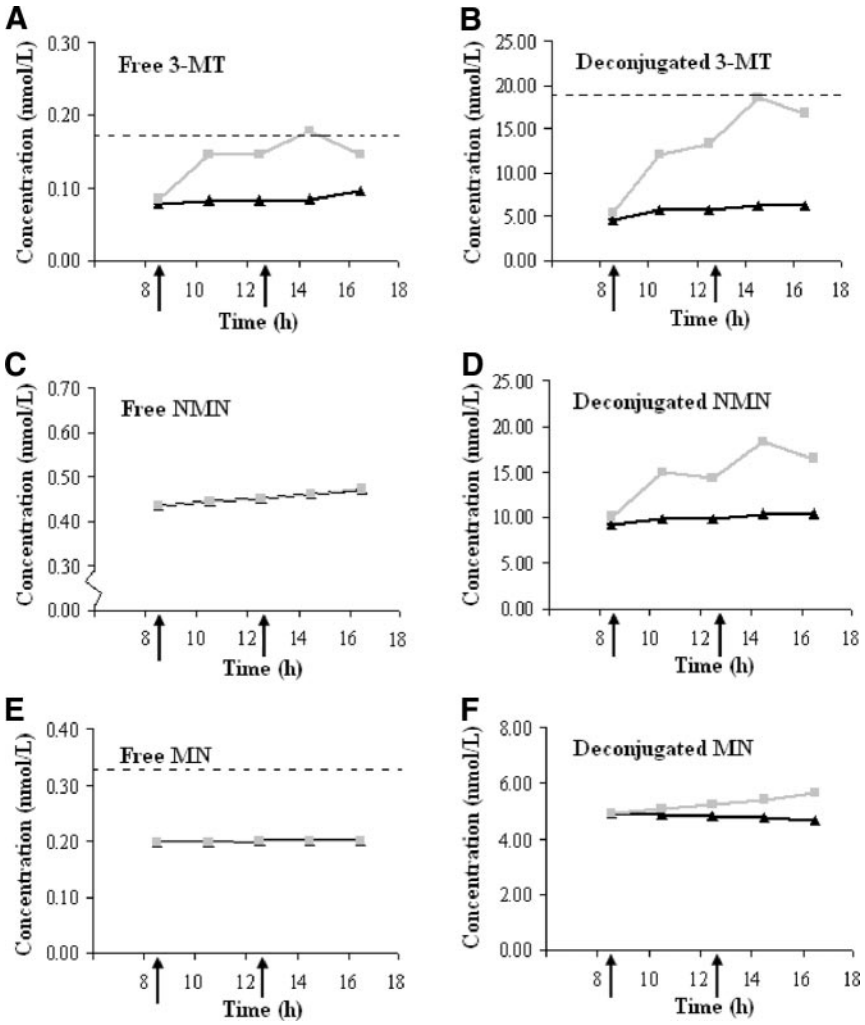

FIG. 3. Line graphs of plasma concentrations of MNs before, during, and after catecholamine-rich and catecholamine-poor meals obtained from statistical linear mixed model fit. Models were calculated from data of 26 subjects in both the high-catecholamine diet and control arms of the study. Changes in concentrations in these models are significant ($P < 0.05$). Control and diet group data are significantly different ($P < 0.05$) from each other when lines do not coincide. The obtained models reflect a true illustration of the raw data (mean ± 95% confidence intervals) (Table 1) after normalization for differences in baseline values (which for the model fit were assumed not to differ on experimental and control arms of the study). A, Plasma free 3-MT; B, plasma deconjugated 3-MT; C, plasma free NMN; D, plasma deconjugated NMN; E, plasma free MN; F, plasma deconjugated MN. *Arrows* indicate the times at which test meals were taken (0830 and 1230 h). *Gray line*, Model for the diet group (n = 26); *black line*, model for the control group (n = 26). Models for plasma free NMN (C) and free MN (E) in diet and control groups are calculated to be similar; therefore both lines coincide. The *dotted line* indicates the upper reference limit of the analyte in the graph.

Influence of diet on urinary NMN

Urinary free NMN showed similar daytime increases and nighttime decreases ($P < 0.05$) during the 24-h period after both the catecholamine-rich and control meals (Fig. 4C). The observed small difference in the model fit after control and catecholamine-rich meals was not significant, indicating no influence of the high-catecholamine diet on urinary free NMN. In contrast to urinary free NMN (Fig. 4C), urinary deconjugated NMN showed significant ($P < 0.05$) differences after ingestion of control *vs.* experimental meals up until the following morning (Fig. 4D). On the day of the catecholamine-rich diet, urinary deconjugated NMN increased by nearly 3-fold over baseline values for the collection between 1430 and 1630 h and remained 2-fold higher for the overnight collection.

Influence of diet on urinary MN

Urinary free (Fig. 4E) and deconjugated MN (Fig. 4F) showed the same time course of changes after both control and experimental meals, indicating no influence of the catecholamine-rich diet. Urinary deconjugated MN, and to a lesser extent urinary free MN, both showed significant ($P < 0.05$) increases during the day, followed by decreases at night.

Discussion

This study shows that dietary catecholamines can dramatically influence plasma as well as urinary concentrations of the 3-O-methylated metabolites of catecholamines. More specifically consumption of catecholamine-rich food products results in sustained and substantial increases in plasma and urinary deconjugated NMN and 3-MT, smaller increases in free 3-MT, but negligible effects on free NMN and MN. These data are consistent with other findings showing that the gastrointestinal tract provides a major site for sulfate conjugation of catecholamines (15, 16), that diet can profoundly influence levels of sulfate conjugates (14, 16–18, 26–28), and that the free MNs have a more rapid circulatory clearance than conjugated metabolites with different sites of production (29). The present study extends these earlier findings by providing novel data about dietary influences on free and deconjugated MNs in urine and plasma, with important implications for biochemical testing of catecholamine-producing tumors.

Our study further shows that whereas the free MNs are relatively insensitive to dietary factors, they are quite sensitive to the conditions of blood sampling, showing rapid decreases within 30 min of supine rest after insertion of an iv catheter. The drops in plasma free NMN and MN after supine rest reflect decreased release of norepinephrine and epinephrine that is consistent with previous findings of posture-associated changes in plasma MNs (20, 30). The lesser effect on MN than NMN is attributable to the substantial amount of circulating MN produced within adrenal medullary cells independently of epinephrine release (4). Lack of influence of supine rest on plasma deconjugated MNs is explained by the much slower circulatory clearance of the sulfate conjugate than the free metabolites (29, 31, 32). The daytime increases and nighttime decreases in circulatory outputs of urinary free and deconjugated NMN and MN are consistent with previous observations (19, 33, 34) that likely reflect increased sympathoadrenal outflow

TABLE 2. Urinary concentrations of MNs before, during, and after ingestion of catecholamine-rich and catecholamine-poor food products

Control	Time						↑ Reference limit
	Morning urine, 0830 h[a]	830–1030 h	1030–1230 h	1230–1430 h	1430–1630 h	1630–0830 h	
3-MT free							46
Control	23.9 (21.3–26.5)	23.8 (21.2–26.3)	26.4 (23.1–29.9)	25.8 (22.3–29.4)	26.7 (23.1–30.3)	22.8 (19.9–25.7)	
Diet	23.1 (20.8–25.4)	28.8 (25.2–32.3)	36.0 (32.1–39.9)	40.3 (34.9–45.7)	37.0 (32.5–41.5)	25.6 (22.9–28.3)	
3-MT deconjugated							197
Control	80 (66–95)	67 (57–78)	81 (67–95)	85 (70–100)	87 (73–101)	80 (68–91)	
Diet	78 (62–95)	81 (67–95)	124 (108–140)	154 (132–176)	165 (146–183)	104 (91–118)	
NMN free							29
Control	11.5 (10.0–13.1)	16.2 (14.3–18.1)	18.3 (15.9–20.6)	17.6 (15.5–19.7)	18.4 (15.6–21.1)	13.1 (11.4–14.7)	
Diet	10.8 (9.2–12.4)	16.8 (15.0–18.6)	19.9 (18.0–21.9)	20.4 (18.4–22.5)	20.4 (18.1–22.8)	16.1 (14.2–18.0)	
NMN deconjugated							260
Control	118 (104–132)	113 (97–129)	124 (107–141)	130 (113–146)	135 (116–153)	125 (109–140)	
Diet	115 (103–128)	122 (109–134)	193 (170–216)	248 (220–276)	304 (269–339)[b]	239 (206–272)	
MN free							20
Control	11.3 (9.7–13.0)	11.9 (10.3–13.4)	13.2 (11.5–14.9)	12.6 (10.9–14.3)	13.6 (11.7–15.6)	11.1 (9.5–12.6)	
Diet	11.4 (9.8–13.0)	12.6 (10.9–14.3)	12.0 (10.5–13.6)	11.9 (10.3–13.5)	12.0 (10.3–13.7)	11.9 (10.2–13.6)	
MN deconjugated							
Control	57 (49–66)	56 (48–64)	63 (53–73)	64 (56–73)	65 (56–74)	58 (50–65)	
Diet	58 (51–66)	60 (52–68)	62 (55–70)	65 (56–74)	65 (57–73)	59 (50–68)	

Test meals containing catecholamines were taken at 0830 and 1230 h; drinks at 1030 and 1430 h. Urinary concentrations are given in micromoles per mole creatinine (n = 26). Data within *parentheses* denote 95% confidence intervals. Deconjugated fractions refer to free + conjugated metanephrines. The first urine collection consisted of first morning urine and all urine excreted after that until 0830 h. Note: Upper limits of reference intervals (Reference limit) were obtained without dietary restrictions, indicating undervalued upper limits. In addition, limits may be distorted because they were determined in 24-h urine collections instead of 2-h and overnight portions. Because of the used model fit statistics, significant changes over time are not expressed in this table.

[a] Baseline time.

[b] Above reference value.

related to a more ambulatory and active status during waking hours.

Lack of effect of the high-catecholamine diet on free NMN in plasma and urine, but the substantial effects on deconjugated NMN, reflect the importance of sulfate conjugation for metabolism of dietary catecholamines (14–16) and the different sources of free and sulfate-conjugated NMN (29). The increases in both free and deconjugated 3-MT after the high-catecholamine diet are also consistent with previous observations of the more sensitive nature of free dopamine and its metabolites to dietary catecholamines (16, 35). The higher amounts of dopamine than of norepinephrine and negligible amounts of epinephrine in food products presumably also contribute to the above differences and lack of influence of diet on plasma and urinary MN.

Catecholamines have a relatively low abundance in food products compared with serotonin. Consequently, dietary restrictions have generally been considered unnecessary for diagnosis of catecholamine-producing pheochromocytomas (13). In recent years, however, there has been a shift in the way these tumors are diagnosed. Rather than focusing on measurements of plasma or urinary free catecholamines, which are relatively insensitive to dietary influences, the focus today is on measurements of plasma or urinary MNs, now recognized to offer superior diagnostic sensitivity compared with the parent amines (1–3, 5–7). Although there have been a few studies indicating an influence of diet on urinary deconjugated MNs (36, 37), others have indicated little influence (19, 38), and until now it has remained unclear whether the conjugated or free metabolites are susceptible to any effect of diet.

The present study is, to our knowledge, the first to investigate the short-term influence of a catecholamine-rich diet on free and deconjugated MNs in plasma and urine using modern analytical techniques characterized by a combination of high sensitivity, specificity, and precision (10). Although the total amounts of specific catecholamine-rich foods (*e.g.* six bananas) ingested may seem unusual from the standpoint of a typical Western diet, it should be appreciated that there are many other food products besides fruits and nuts capable of similar influences (*e.g.* tomatoes, beans, and other vegetables; cheeses; fermented foods; processed meat products). Most are likely unrecognized. In one study involving ingestion of a single ordinary meal, for which there was no consideration of catecholamine content, plasma levels of dopamine sulfate increased by 46-fold with lesser increases in the sulfate conjugates of norepinephrine and epinephrine (16). Similarly large increases in plasma dopamine sulfate were also observed in another study involving ingestion of ordinary meals (26). In that study, contents of catecholamines in one of the three meals that were consumed were more than 10-fold higher than the total amounts of catecholamines consumed in all the meals combined in the present study. Thus, by this standard of an ordinary meal, the total amounts of catecholamines consumed in the present study cannot be considered unusual.

Dietary influences may not, however, be confined to food products that contain catecholamines. Cereals and wheat germ-containing products, which contain negligible amounts of catecholamines, are also capable of eliciting large increases in dopamine sulfate. The mechanism appears to involve the presence of tyrosinase, which converts tyrosine to L-dopa (27). Additionally, tyramine in the diet may be metabolized by mixed-function oxidases and other enzymes to dopamine and 3-MT (39).

With the above in mind, the results of the present study indicate a need to reconsider dietary restrictions during the laboratory diagnosis of catecholamine-producing tumors. Although

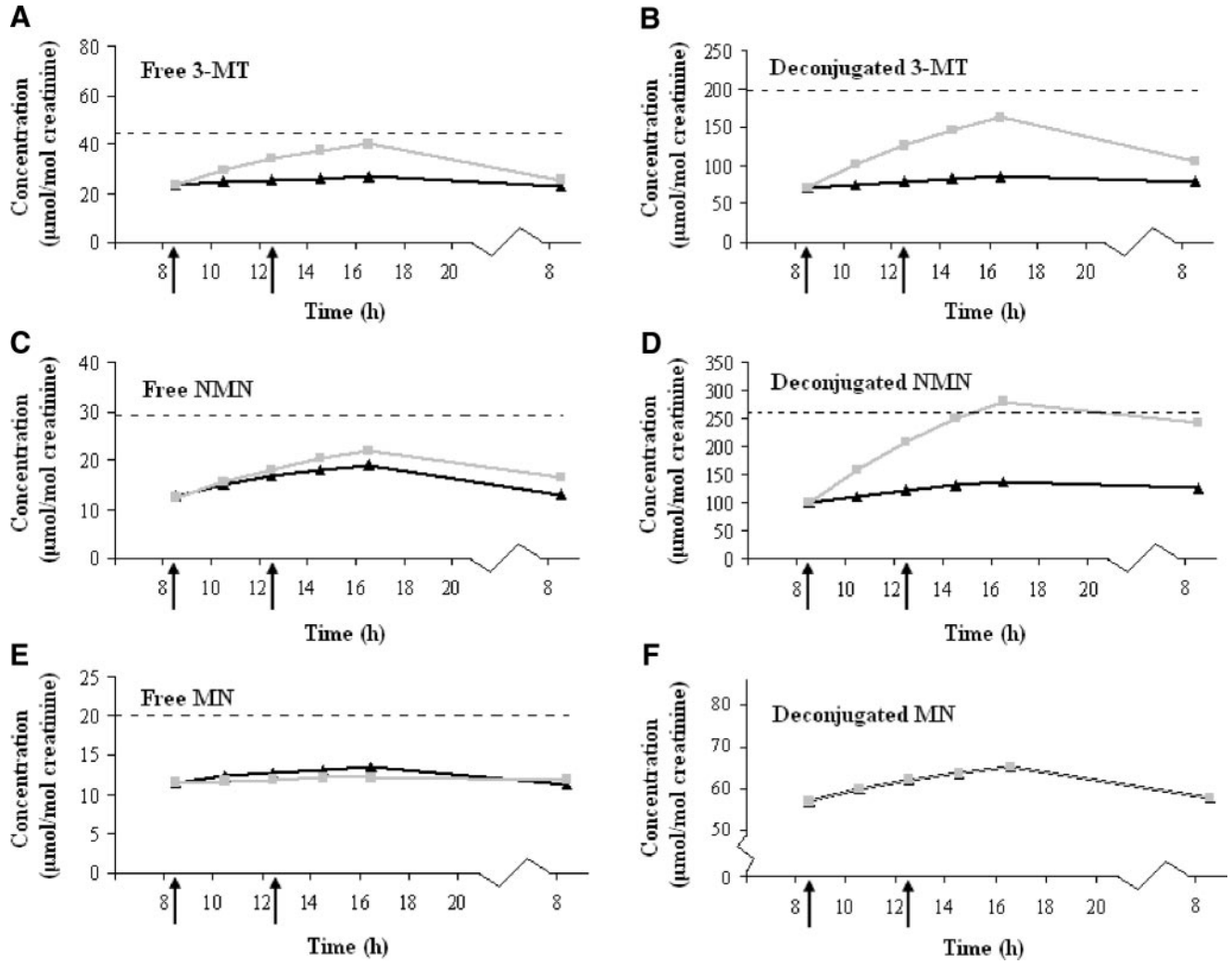

FIG. 4. Line graphs of urinary concentrations of MNs before, during, and after catecholamine-rich and catecholamine-poor meals obtained from statistical model fit. Models were calculated from data of 26 subjects in both the control and the diet group. Changes in concentrations in these models are significant ($P < 0.05$). Control and diet group data are significantly different ($P < 0.05$) from each other when lines do not coincide. The obtained models reflect a true illustration of the raw data (mean ± 95% confidence intervals) (Table 2) after normalization for differences in baseline values (which for the model fit were assumed not to differ on experimental and control arms of the study). A, Urinary free 3-MT; B, urinary deconjugated 3-MT; C, urinary free NMN; D, urinary deconjugated NMN; E, urinary free MN; F, urinary deconjugated MN. *Arrows* indicate the times at which test meals were taken (0830 and 1230 h). *Gray line*, Significant model for the diet group (n = 26); *black line*, significant model for the control group (n = 26). Models for urinary deconjugated MN (F) in diet and control groups are calculated to be similar; therefore both lines coincide.

dietary restrictions appear unnecessary for measurements of free NMN and MN in plasma or urine, our data indicate that such restrictions may be important for measurements of deconjugated NMN, MN, 3-MT, and also free 3-MT in both matrices. The simplest countermeasure to minimize any dietary influence on free 3-MT is an overnight fast, a precaution recommended previously for measurements of plasma free MNs (30). Whether an overnight fast is sufficient for plasma deconjugated MNs is unclear. As illustrated by the incomplete fall in morning urinary deconjugated MNs after the high-catecholamine diet, fasting does not appear sufficient for overnight urine samples and is impractical for 24-h collections. For such collections, avoidance of catecholamine-rich products seems appropriate.

Among three studies of diagnostic tests for pheochromocytoma that included measurements of plasma free and urinary deconjugated NMN and MN (2, 5, 40), all indicated markedly better diagnostic performance of both tests compared with the parent catecholamines and moderately better performance of plasma free than urinary deconjugated NMN and MN. Associated findings of up to 3-fold more false-positive results for urinary deconjugated than free NMN and MN (5) may be related to dietary influences on conjugated but not on free metabolites. This possibility may be explored in future studies employing dietary restrictions before testing for the tumor. Patients with positive urinary deconjugated MN results could be alternatively retested after avoidance of catecholamine-rich food products.

The above suggestion is in line with similar recommendations for plasma free MNs, where findings of positive test results after blood sampling in the seated position should be followed by testing in the supine position (6, 20). Our data support that and the related recommendation that reference intervals for plasma free MNs should be established in samples taken after supine rest (20). By similar reasoning, it also seems appropriate to recommend that reference intervals for urinary and plasma deconju-

gated MNs should be established with consideration of dietary influences.

The reference values for plasma free MNs outlined in the present study were established by blood sampling in the seated rather than the recommended supine position and need to be reestablished. The subsequent lower upper limits would, however, also be expected to result in an increased likelihood of false-positive test results should the appropriate corresponding precautions not be taken during diagnostic testing. However, the relatively easy precautions of an overnight fast plus blood samples collected in the supine position for measurement of free MNs compared with longer-term dietary restrictions required for measurement of deconjugated MNs indicate further advantages for the determination of free over deconjugated metabolites.

Conclusion and recommendations

Catecholamine-rich food consumption has no clinically relevant effect on concentrations of plasma and urinary free NMN, free MN, and deconjugated MN. Therefore these analytes can be determined without preceding dietary restrictions. In contrast, dietary restrictions are indicated for measurements of plasma and urinary free 3-MT, deconjugated 3-MT, and deconjugated NMN. The diet dependency of urinary deconjugated NMN is potentially important because measurements of urinary deconjugated MNs are commonly used for the diagnosis of pheochromocytoma. To improve diagnostic performance, consideration should be given either to employing dietary restrictions or to alternative measurements of urinary or plasma free MNs. Our data also imply that dietary restrictions are necessary for the measurements of 3-MT as a biochemical marker for dopamine-producing pheochromocytomas and neuroblastomas.

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