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Age-specific pediatric reference intervals for plasma free normetanephrine, metanephrine, 3-methoxytyramine and 3-*O*-methyldopa: Particular importance for early infancy

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ABSTRACT

Background: Availability of appropriately established reference intervals for biochemical tests can be troublesome in pediatrics. Here we establish age-specific continuous reference intervals for catecholamine *O*-methylated metabolites in children evaluated for catecholamine producing tumors, particularly younger children with suspected neuroblastoma.

Methods: Plasma concentrations of 3-methoxytyramine, normetanephrine, metanephrine, and 3-*O*-methyldopa were analyzed by liquid chromatography tandem mass spectrometry in 533 children aged 2 days to 18 years.

Results: Concentrations of plasma free normetanephrine, 3-methoxytyramine and 3-*O*-methyldopa were higher in neonates up until six months of age, but thereafter declined steeply to levels after one year that were < 38% those of neonatal concentrations and to further lower concentrations in teenagers that were < 23% those in neonates. In contrast, concentrations of plasma free metanephrine showed a reciprocal pattern with 50% lower concentrations in infants below one year compared to later in childhood.

Conclusion: The dynamic reciprocal changes in plasma concentrations of normetanephrine, 3-methoxytyramine and 3-*O*-methyldopa compared to metanephrine during early childhood suggest underlying developmental changes in extra-adrenal and adrenal chromaffin tissue that must be considered for pediatric reference intervals, particularly in infants. With such reference intervals at hand, biochemical testing for catecholamine producing tumors in young children is substantially improved.

1. Introduction

For first line screening of patients in whom catecholamine producing tumors are suspected, such as pheochromocytomas and paragangliomas (PPGLs), the Endocrine Society recommends measurements of plasma free or urinary fractionated normetanephrine and metanephrine the respective *O*-methylated metabolites of norepinephrine and epinephrine [1]. Additional measurements of 3-methoxytyramine, the *O*-methylated metabolite of dopamine, are useful for diagnosis of dopamine-producing PPGLs [2–4]. More recent reports have suggested that plasma or urinary measurements of these metabolites, but particularly 3-methoxytyramine and normetanephrine, may be similarly useful for diagnosis of neuroblastoma [5–8]. Application of such tests for neuroblastoma is, however, hampered by limited availability of pediatric reference intervals, particularly in early childhood.

Twenty-four hour urinary outputs of catecholamine metabolites show consistent increases with advancing age during childhood, usually accounted for by cut-offs partitioned according to age group [9,10]. Since 24-h urine collections in children can be unreliable and are not feasible in neonates, the commonly employed approach is collection of spot urines with a correction for dilution using creatinine [11–14]. Because creatinine outputs vary according to several factors, particularly lean muscle mass, outputs corrected for creatinine are lower in females than males and also decrease throughout childhood partly as a consequence of gains in muscle mass [11]. The aforementioned issues complicate interpretation of urinary tests of catecholamine excess. Of more importance for catecholamine producing tumors, particularly for PPGLs but potentially also for neuroblastomas, are findings that urinary

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methoxytyramine provides an inferior biomarker for dopamine-producing tumors compared to plasma methoxytyramine [15,16]. More than 90% of urinary dopamine is derived from renal uptake and decarboxylation of circulating 3,4-dihydroxyphenylalanine (L-dopa) [17,18]. Similarly it seems that substantial amounts of urinary 3-methoxytyramine are also derived from sources other than circulating 3-methoxytyramine [16].

For the above reasons our interest has focused on the plasma metabolites, particularly plasma methoxytyramine, which as a biomarker of dopamine-producing tumors should be useful for neuroblastoma. The present study follows that of Franscini and colleagues [5], who examined plasma concentrations of normetanephrine, metanephrine and methoxytyramine in 188 children. Our study builds on that with a larger population of 533 children and use of an LC-MS/MS method that provides higher analytical sensitivity for methoxytyramine than that used previously. Furthermore, our LC-MS/MS method includes measurements of 3-*O*-methyldopa (3-methoxytyrosine, 3-methoxy-4-hydroxyphenylalanine), the *O*-methylated metabolite of L-dopa. The latter immediate precursor of dopamine has been reported as a biomarker of neuroblastoma [19,20], particularly in those tumors with an unfavorable prognosis [21], providing the incentive for inclusion of 3-*O*-methyldopa in the presently reported plasma panel.

2. Methods

2.1. Subjects

Five hundred and thirty three children (265 females), aged 2 days to 17.9 years, were enrolled under the PRIMMS (Pediatric Reference Intervals for Monoamine Metabolites and Steroids) study protocol. Inclusion, either as in-patients or out-patients at the Children's University Hospital Dresden, was according to several criteria: (i) blood sampling was carried out as part of routine clinical care; (ii) the additional volume of blood collected for research purposes did not involve foreseeable additional risk for the child; (iii) children had to be conscious, not in any visible physiological or emotional distress or suffering any acute life-threatening injury or illness; and (iv) enrollment required presence of a legally responsible parent or guardian capable of making informed decisions concerning the child's welfare. An additional group of healthy volunteer adolescents aged 13 to 17 years (71 from the 163 enrolled within this age group), up to but not at or beyond 18 years, were recruited by advertising in local schools. The volunteers of this group had to have read and understood the consent documents. Children who required medications known to interfere with catecholamine systems as well as children with catecholamine producing tumors, such as neuroblastoma, were excluded from the reference group. All children were enrolled under an Ethics committee approved clinical protocol (Ethics committee approval EK 113042013) with signed parental consent and where appropriate verbal ascent or additional signed consent from teenaged children.

2.2. Preanalytics and biochemical analyses

Heparinized blood samples were drawn in the morning and wherever possible after at least 20 min of supine rest, and usually after an overnight fast. For children in whom fasting before blood sampling was not possible (i.e. neonates and younger children), a restricted diet including breast milk, formula, milk, white bread, butter, pure rice waffles, boiled rice as well as pasta was permitted. Blood samples were immediately placed on ice and transferred to the laboratory. Blood samples were centrifuged for 15 min at 4500 rpm and plasma aliquots stored at −80 °C until analyses.

Concentrations of plasma free 3-methoxytyramine, normetanephrine and metanephrine were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described [22,23]. Testing was extended to include 3-*O*-methyldopa. 3-*O*-methyldopa was purchased from Sigma-Aldrich (catalog number 1420006) and its deuterated 3-*O*-methyldopa internal standard (3-methoxy-d3-L-tyrosine) was purchased from Medical Isotopes (catalog number OD74870). For detection and quantification of 3-*O*-methyldopa multiple reaction monitoring transitions for the analyte and the internal standard were respectively optimized to mass to charge ratios of *m/z* 212.1- > 166.1 and 212.1- > 149.1 as quantifier and qualifier ion, and m/z of 215.1- > 198.1. Other conditions remained the same as previously described [23].

2.3. Statistical analysis

Age-specific reference intervals were derived using fractional polynomial models, as described elsewhere [24] and also employed by Franscini et al. [5]. The general procedure is described in the supplemental method 1. In brief, the reference population was separated into equally sized age groups and data for *O*-methylated metabolites normalized by logarithmic transformation. Means and standard deviations for age groups were then used for fitting two independent fractional polynomial models for each metabolite and the estimation of best-fit polynomial coefficients. Following the estimation of polynomial coefficients for means (M(Age)) and standard deviations (SD(Age)), curves of the n^{th} percentile of each analyte were calculated according to the formula:

$$y_n = M(Age) + SD(Age) \bullet \Phi^{-1}\left(\frac{n}{100}\right)$$

where Φ^{-1} is the reverse of the standard normal distribution. Age was previously transformed in all cases as indicated by Royston and Wright [24] according to the formula:

$$t_{age} = e^{\frac{\log(0.01) \bullet (Age - \min(Age))}{(\max(Age) - \min(Age))}}$$

In order to provide practical equations for determination of age-adjusted reference intervals a meta-analysis of the obtained percentile values was performed by fitting a regression line to the derived percentile values for ages of patients. Curve fitting of the percentiles was carried out using Matlab (Matlab version 2015a, Curve fitting tool). Fractional polynomial analysis was conducted using RStudio version 0.99.082–2016 (R version 3.2.1, R Core Team, 2013, http://www.r-project.org/) and the R package mfp. Non-parametric statistical methods were used for comparisons of data using the JMP® PRO software package 12.1.0.

3. Results

3.1. Influence of sex

Among the reference population, girls were older than boys (median ages 9.9 years vs. 7.2 years, $P < .0001$) and median concentrations of plasma free 3-methoxytyramine, normetanephrine and metanephrine were respectively 20%, 35% and 16% higher ($P < .0001$) in boys than in girls (Table 1). Within different age classes (Table 1) those differences remained significant for normetanephrine in children older than 3 years but younger than 6 years ($P < .0001$) and in adolescents older than 13 years but younger than 18 years ($P = .0072$). For metanephrine the differences remained significant in all groups of children between 6 and 18 years ($P < .02$).

After correction for age by multivariate analysis the difference between boys and girls for 3-methoxytyramine was no longer significant (Table 2). For normetanephrine the higher plasma concentrations in boys than girls, although remaining significant ($P < .0001$), were reduced to an 18% difference. For plasma metanephrine differences between males and females were negligibly influenced after correction for age (17% higher values after correction). For 3-*O*-methyldopa no differences between girls and boys were observed over the entire age

Table 1
Medians and observed ranges of plasma free normetanephrine, metanephrine, 3-methoxytyramine and 3-*O*-methyldopa according to sex and age in the reference population.

	N	**Age** (range)	**NMN** (range) [pmol/L]	**MN** (range) [pmol/L]	**MTY** (range) [pmol/L]	**3OMD** (range) [nmol/L]
All subjects	**533**	8.7y (0.005 - 17.9)	404.0 (87.4 - 2970.2)	197.7 (< 20.3 - 775.7)	35.9 (< 23.9 - 789.4)	87.2 (30.5 - 667.1)
Girls	265	9.9y [a] (0.005 - 17.9)	349.4 [a] (87.4 - 2642.6)	182.5 [a] (< 20.3 - 745.3)	35.9 [a] (< 23.9 - 789.4)	82.8 (30.5 - 667.1)
Boys	268	7.2y (0.005 - 17.7)	469.6 (98.3 - 2970.2)	212.9 (< 20.3 - 775.7)	41.9 (< 23.9 - 412.6)	87.2 (30.5 - 536.3)
1 to 30 days	**29**	3d (2 - 17)	1392.3 [e] (556.9 - 2970.2)	147.0 [e] (< 20.3 - 415.7)	167.4 [e] (< 23.9 - 789.4)	335.7 [e] (122.1 - 667.1)
Girls	10	3d (2 - 10)	1403.2 (556.9 - 2642.6)	147.0 (< 20.3 - 380.3)	215.3 (< 23.9 - 789.4)	353.2 (183.1 - 667.1)
Boys	19	3d (2 - 17)	1408.7 (737.1 - 2970.2)	147.0 (< 20.3 - 415.7)	167.4 (< 23.9 - 412.6)	331.4 (122.1 - 667.1)
1 to < 6 months	**22**	2.3m (1.2 - 5.9)	1184.8 [e] (524.2 - 2440.6)	111.5 [e] (< 20.3 - 775.7)	95.7 [e] (< 23.9 - 233.2)	300.8 [e] (139.5 - 549.4)
Girls	7	2.5m (1.5 - 3.8)	846.3 (524.2 - 2440.6)	142.0 (65.9 - 223.1)	95.7 (< 23.9 - 149.5)	318.3 (222.4 -549.4)
Boys	15	2.2m (1.2 - 5.9)	1375.9 (633.4 - 2020.2)	106.5 (< 20.3 - 775.7)	95.7 (< 23.9 - 233.2)	257.2 (139.5 - 440.4)
6 to < 12 months	**18**	9.9m (6.3 - 11.8)	764.4 [e] (480.5 - 1583.4)	218.0 [e] (116.6 - 491.8)	83.7 [e] (< 23.9 - 161.5)	157.0 [e] (65.4 - 313.9)
Girls	6	7.2m (6.6 - 11.5)	1015.6 (600.6 - 1583.4)	218.0 (167.3 - 314.3)	89.7 (< 23.9 - 149.5)	148.2 (65.4 - 209.3)
Boys	12	10.5m (6.3 - 11.8)	737.1 (480.5 - 1184.8)	207.9 (116.6 - 491.8)	83.7 (< 23.9 - 161.5)	157.0 (122.1 - 313.9)
1 to < 3 years	**72**	1.9y (1.0 - 2.9)	524.2 [e] (196.6 - 1425.1)	218.0 [e] (< 20.3 - 501.9)	59.8 [e] (< 23.9 - 149.5)	91.6 [e] (43.6 - 318.3)
Girls	30	2.2y (1.1 - 2.9)	507.8 (196.6 - 1190.3)	197.7 (121.7 - 431.0)	59.8 (29.9 - 131.6)	91.6 (48.0 - 200.6)
Boys	42	1.7y (1.0 - 2.9)	551.5 (294.8 - 1425.1)	233.2 (< 20.3 - 501.9)	59.8 (< 23.9 - 149.5)	100.3 (43.6 - 318.3)
3 to < 6 years	**58**	4.6y (3.1 - 5.9)	393.1 [e] (169.3 - 1403.2)	233.2 [e] (45.6 - 542.5)	41.9 [e] (< 23.9 - 143.5)	100.3 [e] (43.6 - 209.3)
Girls	31	4.3y (3.0 - 5.9)	322.1 [a] (169.3 - 649.7)	228.2 [c] (45.6 - 542.5)	41.9 (< 23.9 - 77.7)	109.0 (43.6 - 209.3)
Boys	27	4.6y (3.1 - 5.9)	491.4 (169.3 - 1403.2)	278.9 (131.8 - 532.4)	35.9 (< 23.9 - 143.5)	95.9 (56.7 - 161.3)
6 to < 13 years	**171**	9.6y (6.0 - 12.9)	360.4 [c] (131.0 - 1277.6)	212.9 [c] (25.4 - 745.3)	35.9 [c] (< 23.9 - 131.6)	82.8 [e] (30.5 - 174.4)
Girls	80	9.4y (6.0 - 12.9)	338.5 (131.0 - 1277.6)	202.8 [d] (86.2 - 745.3)	35.9 (< 23.9 - 83.7)	91.6 (34.9 - 170.0)
Boys	91	10.0y (6.0 - 12.9)	382.2 (131.0 - 922.7)	238.3 (25.4 - 552.6)	35.9 (< 23.9 - 131.6)	82.8 (30.5 - 174.4)
13 to < 18 years	**163**	15.4y (13.1 - 17.9)	322.1 [c] (87.4 - 775.3)	167.3 [c] (45.6 - 385.3)	23.9 [c] (< 23.9 - 155.5)	74.1 [e] (30.5 - 178.8)
Girls	101	15.6y (13.1 - 17.9)	305.8 [c] (87.4 - 775.3)	157.2 [b] (45.6 - 385.3)	23.9 (< 23.9 - 83.7)	74.1 (30.5 - 178.8)
Boys	62	14.9y (13.1 - 17.9)	360.4 (98.3 - 753.5)	182.5 (60.8 - 365.0)	29.9 (< 23.9 - 155.5)	74.1 (34.9 - 178.8)

[a] indicate differences between girls and boys ($P \leq .0001$).
[b] indicate differences between girls and boys ($P \leq .001$).
[c] indicate differences between girls and boys ($P \leq .01$).
[d] indicate differences between girls and boys ($P < .05$).
[e] indicates differences between age groups (tested by nonparametric comparisons for each pair using the Wilcoxon Method). Nine and 114 of 533 values of MN and MTY, respectively, were determined below the limit of quantification (20.3 pmol/L and 23.9 pmol/L, respectively). For MN those belong to age groups 1–30 days ($n = 7$), 1 month to < 6 months ($n = 1$) and 1 year to < 3 years (n = 1). For MTY, 4, 3, 4, 2, 5, 25 and 59 values were observed below the limit of quantification in the respective age groups. For further details please refer to the suppl. data 1.

Table 2
Multivariate analysis of impact of sex and age on plasma concentrations of *O*-methylated metabolites.

	Sex			Age		
Metabolite	F-ratio	P-value	Impact	F-ratio	P-value	Impact
3-Methoxytyramine	2.7	0.1016	none	209.6	< 0.0001	−ve
Normetanephrine	15.6	< 0.0001	M > F	220.0	< 0.0001	−ve
Metanephrine	13.0	0.00003	M > F	6.7	0.0101	−ve
3-*O*-methyldopa	0.5	0.4824	none	218.7	< 0.0001	−ve

Where significant (i.e., P < .05) impacts of sex, where significant, are shown according to higher values in males (M) or females (F) whereas impacts of age are shown according to the negative (−ve) nature of relationships. Data for metabolites were logarithmically transformed before least squares multivariate analyses.

range. Despite the remaining significant differences for normetanephrine and metanephrine in boys versus girls, the 2.5 and 97.5 percentiles, as commonly used as lower and upper reference interval limits showed negligible differences according to sex in contrast to the highly dynamic changes with age.

3.2. Influence of age

The highly dynamic age-related changes in plasma concentrations of *O*-methylated metabolites were most pronounced up until the age of three (Fig. 1, Table 1). These changes were characterized by reciprocal fall with age for 3-methoxytyramine, normetanephrine and 3-*O*-methyldopa compared to an increase for metanephrine. Median plasma concentrations of 3-methoxytyramine, normetanephrine and 3-*O*-methyldopa fell ($P < .0001$) steeply with age to respective values of 36%, 38% and 27% in children at three years of age compared to neonates; concentrations then fell further more slowly to 23%, 14% and 22% in teenagers compared to neonates (Table 1). Negative relationships of those three *O*-methylated metabolites with age remained highly significant ($P < .0001$) after correction for sex differences by multivariate analysis (Table 2). In contrast, plasma concentrations of metanephrine were lower in neonates and infants up to 6 months compared to older children ($P < .0001$), then gradually fell from 5 years of age throughout adolescence (Fig. 1). By multivariate analysis there was a weak negative relationship ($P = .0101$) of plasma metanephrine concentrations with age, this reflecting gradual drop in concentrations after the age of five.

The dynamic age-related changes in metabolite concentrations necessitated construction of age-specific continuous reference intervals. For this fractional polynomial analysis was used to estimate the polynomial coefficients of the mean and standard deviations along with the coefficients, standard errors and *P*-values for each analyte (Suppl. Methods 1). Based on the estimated coefficients, age-dependent reference curves for 2.5, 25, 50, 75 and 97.5 percentiles were formulated (Fig. 1). Subsequently, a regression line was generated based on the percentile values. For all cases, regression line R^2 values were equal or above 0.97, assuring the validity of the generated equations. The detailed procedures for extracting percentiles and percentile curves for all analytes are presented in supplemental information (Suppl. Methods 1). Percentile curve regression line derived equations for determination of continuous age-specific reference intervals for 3-*O*-methyldopa, 3-methoxytyramine, normetanephrine, and metanephrine are provided in Table 3 as a function of age (x in years).

Applying those aforementioned equations to calculate upper limits of reference intervals, as defined by the 97.5 percentiles of data distributions, upper limits of reference intervals for concentrations in

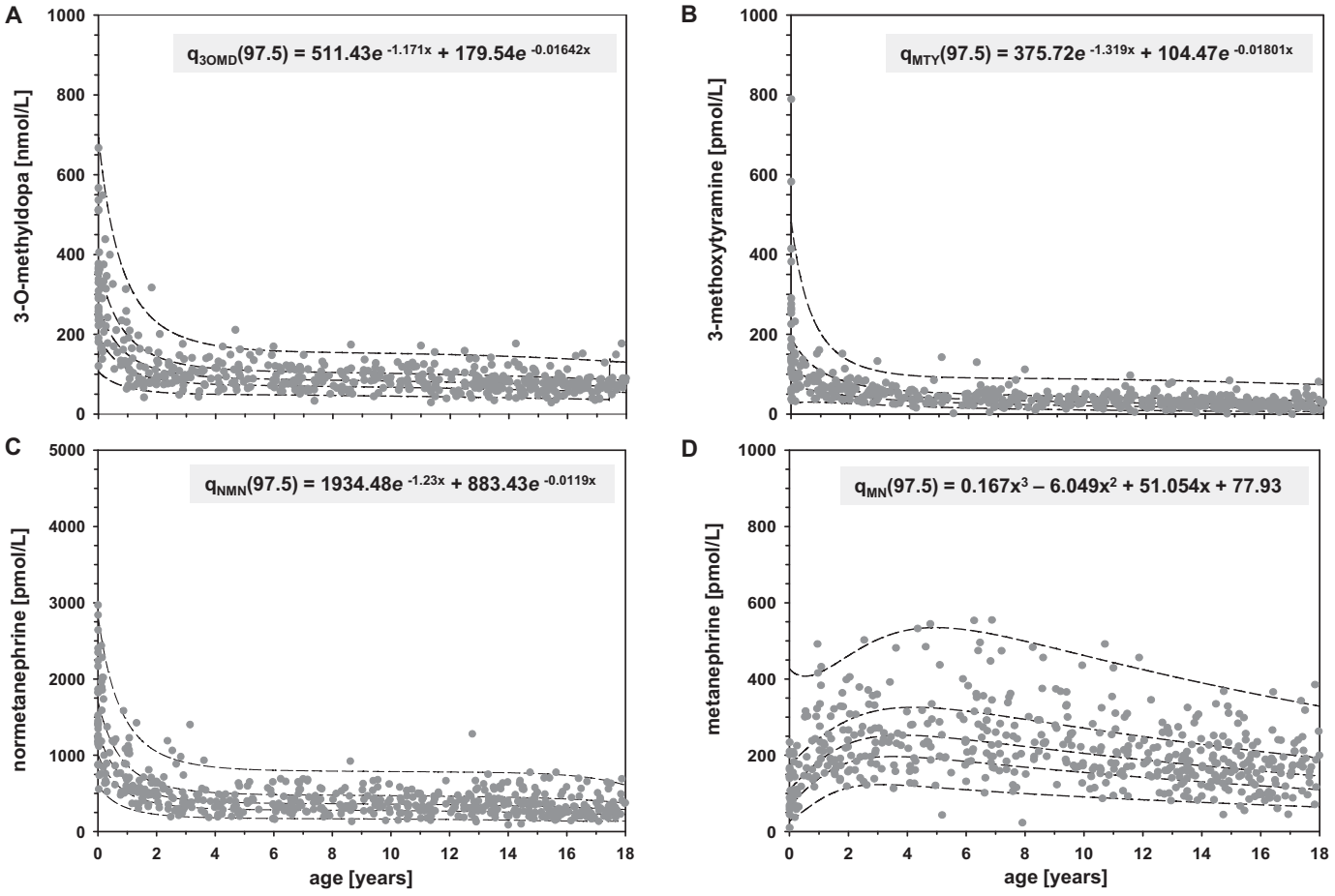

Fig. 1. Relationships of age and concentrations of 3-*O*-methyldopa (A), 3-methoxytyramine (B), normetanephrine (C) and metanephrine (D) for neonates to 18 year old children in the reference population. Dashed lines represent the 2.5, 25, 50, 75 and 97.5 percentiles. Equations given in A, B, C and D should be used to calculate age-specific upper limits of reference intervals (97.5 percentiles).

neonates to teenagers ranged from 686 nmol/L to 179 nmol/L for 3-*O*-methyldopa, from 479 pmol/L to 78 pmol/L for 3-methoxytyramine and from 2811 pmol/L to 737 pmol/L for normetanephrine (Table 3). Much smaller differences in upper limits of reference intervals were determined for metanephrine (Table 3).

4. Discussion

This study provides comprehensive data on distributions of plasma concentrations of 3-methoxytyramine, normetanephrine, metanephrine and 3-*O*-methyldopa in a pediatric population of more than 530 children ranging from neonates to adolescents. Pronounced decreases in plasma concentrations of methoxytyramine, normetanephrine and 3-*O*-methyldopa with advancing age, in association with reciprocal increases in plasma metanephrine, revealed the importance of age-specific reference intervals in neonates and infants versus older children. To address this need, equations were established using curve fitting for provision of reference intervals specific for children of any age from newborns to teenagers.

Although a population size of 120 subjects is usually sufficient for establishing reference intervals [25], this can be inadequate when distributions of test results vary with sex or age, the latter as shown here for plasma metabolites and elsewhere particularly important for urinary metanephrines in children [12–14]. Establishing reference

Table 3
Equations for determination of age-specific upper limits of reference intervals of *O*-methylated metabolites, examples.

	1d	15d	30d	3m	6m	9m	12m	2y	3y	15y
3-methyldopa										
$q_{97.5} = 511.43e^{-1.171x} + 179.54e^{-0.01642x}$ [nmol/L]	686	663	641	558	462	388	336	227	192	179
$q_{97.5} = 117.3e^{-1.171x} + 41.18e^{-0.01642x}$ [ng/mL]	157	152	147	128	106	89	77	52	44	41
3-methoxytyramine										
$q_{97.5} = 375.72e^{-1.319x} + 104.47e^{-0.01801x}$ [pmol/L]	479	460	443	377	299	245	203	126	108	78
$q_{97.5} = 62.83e^{-1.319x} + 17.47e^{-0.01801x}$ [pg/mL]	80	77	74	63	50	41	34	21	18	13
Normetanephrine										
$q_{97.5} = 1934.48e^{-1.23x} + 883.43e^{-0.0119x}$ [pmol/L]	2811	2725	2632	2304	1922	1643	1436	1026	901	737
$q_{97.5} = 354.3e^{-1.23x} + 161.8e^{-0.0119x}$ [pg/mL]	515	499	482	422	352	301	263	188	165	135
Metanephrine										
$q_{97.5} = 0.167x^3 - 6.049x^2 + 51.054x + 395.1$ [pmol/L]	395	395	401	406	421	431	441	477	497	370
$q_{97.5} = 0.0333x^3 - 1.193x^2 + 10.07x + 77.93$[pg/mL]	78	78	80	80	83	85	87	94	98	73

intervals for pediatric blood tests is however a challenge due to ethical and consensual practicalities that can severely constrain recruitment of sufficiently sized populations of children, particularly neonates and infants.

The earliest study focusing on pediatric plasma normetanephrine and metanephrine was limited to 86 children [26], but these were all over 5 years of age. Although the present study confirms that sex has a significant impact on plasma metanephrine, with higher concentrations in males than females also found in adults [27], the earlier study in children [26] failed to establish any dynamic age-related changes in concentrations of plasma metabolites throughout childhood. Concentrations of metanephrine were shown to be higher in children than adults, whereas those of normetanephrine were lower in children than adults, the latter in keeping with age-related increases extending into adulthood [27].

A subsequent study by Franscini et al. [5] that included neonates and infants was the first to reveal the highly dynamic and reciprocal age-related changes in plasma free normetanephrine and metanephrine in early childhood, findings that are confirmed here. The present series extends these earlier findings using a nearly 3-fold larger pediatric population and additional measurements of 3-*O*-methyldopa, the *O*-methylated metabolite of L-dopa. More importantly, enhanced analytical sensitivity of our LC-MS/MS method allowed for measurements of plasma free 3-methoxytyramine, which could not be reliably detected in the reference population of Franscini et al. [5]. We thereby establish, similar to normetanephrine, that plasma concentrations of both 3-methoxytyramine and 3-*O*-methyldopa show substantial decreases in early childhood that must also be considered for establishing pediatric reference intervals.

Although involving 533 children, this population remained insufficiently sized to calculate reference intervals for specific age groups from neonates to infants with either traditional use of 95% confidence intervals or non-parametric-derived 2.5 and 97.5 percentiles. We therefore followed the approach of Franscini et al. [5], in which fractional polynomial analyses enables derivation of formulae for calculation of continuous age-specific reference intervals. This is also an approach we have adopted for age-specific reference intervals for plasma free normetanephrine and steroids in adult populations [27,28]. The curves and equations established here for normetanephrine and metanephrine are in good agreement with those defined by Franscini et al. [5], whereas those for methoxytyramine and 3-*O*-methydopa are described here for the first time.

The dramatic decreases in plasma methoxytyramine, normetanephrine and 3-*O*-methyldopa in neonates to infants up and until about 3 years of age illustrates the importance of age-specific reference intervals for these metabolites, particularly in early childhood. Although screening for pheochromocytomas and paragangliomas in children with hereditary predisposition for these tumors is not recommended until after 5 years of age there have been isolated reports of children with these tumors at much earlier ages [29–31]. Of potentially more importance is the application of the currently described reference intervals to children with neuroblastoma, which mostly occur in infancy. For such tumors, preliminary data suggest that measurements of plasma methoxytyramine and normetanephrine may be particularly promising, whereas measurements of metanephrine appear unhelpful [5].

Measurements of 3-*O*-methyldopa have been proposed for newborn screening of L-aromatic amino acid decarboxylase deficiency [32,33], with measurements in urine also proposed as a prognostic biomarker for neuroblastoma [34]. The latter proposal is in line with reports that L-dopa, the amino acid precursor of 3-*O*-methyldopa, may have prognostic value as a biomarker for neuroblastoma [19–21]. Moreover urinary methoxytyramine, which is in part derived from intra-renal *O*-methylation of dopamine produced after renal decarboxylation of circulating L-dopa [35], also appears useful as biomarker of aggressive neuroblastoma [8]. Together the aforementioned findings provide relevance for the present provision of reference intervals for plasma free 3-methoxytyramine, 3-*O*-methydopa and normetanephrine in newborns, infants and older children. With this diagnostic roadblock removed it is now possible to apply these measurements as a potential tool for biochemical testing and management of children with neuroblastoma.

The reciprocal early infancy increases in plasma free metanephrine compared to decreases in 3-methoxtyramine, normetanephrine and 3-*O*-methyldopa are not only in agreement with Franscini et al. [5], but also other comparisons in early infancy and later childhood of urinary excretion of epinephrine and metanephrine relative to norepinephrine, dopamine and their metabolites [36]. It is possible that an early developmental switch in tyrosinase-related production of dopamine to melanin [37] might contribute to early infancy-related changes of methoxytyramine and 3-*O*-methyldopa. More likely, however, is that the large drops in plasma 3-*O*-methyldopa, 3-methoxytyramine and normetanephrine in early infancy reflect postnatal apoptosis of neural crest-derived para-aortic chromaffin tissue, such as in the organ of Zuckerkandl [38,39], which provides potential sources for those metabolites. In contrast, plasma concentrations of metanephrine reflect the size of adrenal medullary stores of epinephrine and depends on glucocorticoid-induced induction of phenylethanolamine *N*-methyl transferase (PNMT), the enzyme responsible for transformation of norepinephrine to epinephrine. The fetal to postnatal changes in functions of intra- and extra-adrenal chromaffin cells are conceived to reflect changes in regulation of blood pressure and oxygenation before and after birth [26].

5. Conclusion

With the availability of age-specific continuous reference intervals for plasma free normetanephrine, 3-methoxytyramine, 3-*O*-methyldopa and metanephrine, a prerequisite for test interpretation, a major roadblock has been removed for access of pediatric populations to the same diagnostic strategies and technologies available to adults. It is thus now possible to bring laboratory testing for catecholamine producing tumors, such as neuroblastoma and childhood pheochromocytoma, in pediatric populations up to the same level of quality already achieved for diagnosis of chromaffin cell tumors in adult populations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.03.1620.

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