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The effect of phlebotomy and placement of an intravenous catheter on plasma catecholamine and serum copeptin concentrations

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Abstract

Objectives: Limited data are available on the hormonal response of children to venepuncture or intravenous cannulation (IVC). Catecholamines (epinephrine (E) and norepinephrine (NE)) have been traditionally recognized as stress hormones. Copeptin, the carboxyl-terminus of the arginine vasopressin (AVP) precursor peptide, is also a known marker for stressful stimuli, including myocardial infarction, critical illness, and sepsis. We aimed to measure the above stress markers in response to IVC in the pediatric population.

Methods: We measured plasma E, NE and serum copeptin concentrations in 100 children aged 5–17 years undergoing endocrine testing. Labs were drawn 1–3 min (min) after placement of IV cannula (baseline or 0 min) and then re-measured 20 min later (+20 min) while subjects rested in a quiet room.

Results: Between 0 and 20 min, the median (IQR) NE (n=99) changed from 349 (244, 482) pg/mL to 253 (184, 348) pg/mL (p<0.001); E (n=54) changed from 57 (43, 116) pg/mL to 57 (38, 96) pg/mL (p=0.024); Copeptin changed from 9.4 (6.3, 15.2) pmol/L to 9 (5, 13) pmol/L (p<0.001). The mean decrease (delta) was 106 pg/mL for NE (28 %, p<0.001), 16 pg/mL for E (18 %, p=0.042) and 2.7 pmol/L for copeptin (17 %, p=0.012). There was no correlation between the decrease (expressed as a percentage) in NE vs. E, E vs. copeptin, and NE vs. copeptin.

Conclusions: Our data suggest that the stress of IVC induces a rapid increase in NE, E, as previously described, as well as copeptin levels. The copeptin decrement, concordant with the catecholamine trend in the minutes after IVC, supports this peptide (and AVP) as a rapid response marker of stress, and has unclear practical implications for copeptin measurements in evaluating fluid and sodium metabolism disorders in children.

Keywords: norepinephrine; epinephrine; pain; venipuncture; pediatrics; copeptin

Introduction

Venepuncture and IV cannula insertion are the most common causes of stress and pain in the medical setting for the pediatric population [1]. Although stress hormone levels are presumed to rise in the minutes following intravenous cannulation (IVC), limited data are available in this regard.

Catecholamines (epinephrine, norepinephrine, and dopamine), have been traditionally described as stress hormones and shown to increase in settings of physical pain and mental stress. Epinephrine and metanephrine are primarily secreted by the adrenomedullary chromaffin cells, while most of the circulating norepinephrine and normetanephrine are released by the synapses of sympathetic nerves.

Arginine vasopressin (AVP), also known as antidiuretic hormone, is co-released with copeptin in a one-to-one equimolar ratio from the posterior pituitary into the systemic circulation in response to hemodynamic and osmotic stimuli primarily to preserve vascular tone (via the V1a receptors, V1a-R) and fluid homeostasis (via the V2-R). A second neurosecretory pathway delivers AVP from parvocellular neurons through the portal vessels to the anterior pituitary, where it acts synergistically with corticotropin-releasing

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hormone (CRH) to stimulate adrenocorticotropic hormone (ACTH) and subsequently cortisol synthesis in response to stressors (via the V1b-R). Moreover, AVP binding to the V1b-R in the adrenal medulla results in short-term catecholamine secretion [2, 3]. AVP is thought to mediate pain perception through centrally located V1a and V1b receptors [4]. Arginine vasopressin (AVP) is thus recognized as a hypothalamic stress hormone, but its accurate quantitation in plasma is difficult due to pre-analytical and analytical hurdles [5], therefore limiting its use as a stress marker.

On the other hand, copeptin is a stable peptide that can be measured accurately by a robust immunometric assay [6], and as a reliable surrogate of AVP, can be utilized as a stress marker. Previous studies demonstrated elevated copeptin levels in the settings of myocardial infarction, sepsis, and other conditions of acute stress [7]. However, limited data are available on circulating copeptin levels and their relationship with catecholamine levels in the context of painful stimuli.

This study aimed to assess the response of catecholamines and copeptin to IVC in a cohort of healthy children.

Subjects and methods

A convenience sample of 100 children and adolescents who presented to the UPMC Children's Hospital of Pittsburgh's outpatient Endocrinology testing unit was included in the study. Subjects were scheduled to undergo a growth hormone stimulation test or other endocrine tests (i.e., oral glucose tolerance test, ACTH stimulation test, Leuprolide stimulation test) as part of their routine clinical care. The subjects were evaluated for conditions such as short stature, possible glucose intolerance, and adrenal or pubertal disorders. They were otherwise healthy with no known history of ongoing medical issues such as electrolyte abnormalities, chronic pain syndromes, or inflammatory conditions. Most were on no medications; a few patients were taking medications known not to interfere with the assay for E or NE. Written informed consent (and assent from children 10 years or older) was obtained from the legal representatives of each subject and participant, respectively, before participation. This study was approved by the Institutional Review Board of the University of Pittsburgh (PRO19120068).

After an overnight fast, the subjects arrived at the testing room and lay in a resting, semi-recumbent position, with the head and chest about 45° above the horizontal line. This position was selected after several attempts as the most comfortable and agreeable for children who consistently wanted to watch the IVC procedure and would not accept lying supine in most instances. They were kept in this same position from 10 min before IVC placement until the collection of 2 blood samples for this study was completed, as detailed below. IVC was performed by highly trained nurses in our endocrinology testing unit, with a 23-G angiocatheter exclusively. Only children with a single successful attempt for IVC using distraction techniques (no topical anesthetics) were included. Blood samples for measuring copeptin, E, NE, and dopamine (DA) were collected before administering any agent by the oral or parenteral route per the test protocol. Each subject had two separate blood collections. The first sample was drawn 1–3 minutes (min) after placement of an IV cannula (0-min or baseline sample). A minimum of 1 min, but generally 90–180 s, was the time needed for the nurses to tape the IV in place and obtain blood for the analytes necessary for hormone testing, after which the samples for copeptin and catecholamines were obtained. We deemed this time interval of 1–3 min after IVC appropriate to allow the secreted copeptin and catecholamines to reach the peripheral circulation from their release site. A second blood sample was obtained 20 min later (20-min sample) from the indwelling IV cannula.

Blood for catecholamines was drawn in chilled heparinized tubes, kept on ice, and promptly spun. The serum for copeptin was separated after spontaneous clotting of the blood samples. Plasma and serum samples were immediately frozen and kept at −80 °C until analyzed. Samples were analyzed at Quest Laboratories-Nichols Institute, San Juan Capistrano, CA.

E, NE, and DA concentrations were measured in the plasma using HPLC + electrochemical detection (HPLC-ED) for the first 86 subjects (172 samples), and by LC/MS/MS (LCMS) for the last 14 subjects (28 samples) due to a change in methodology at the laboratory. When samples were analyzed and compared with both methodologies in a different subset of subjects (n=170) for study validation purposes, a good to excellent correlation between the two methods was demonstrated. Below, we indicate the equations to convert the results obtained via LCMS indicated by y) methods to the corresponding HPLC-ED (indicated by x) values.

Norepinephrine: $y=1.081*x-43.40$ ($R^2=0.92$)

Epinephrine; $y=1.040*x-7.74$ ($R^2=0.75$)

Dopamine: $y=0.8656*x-3.303$ ($R^2=0.65$).

The limit of quantitation (LOQ) for the HPLC-ED assay was 20 pg/mL for both E and NE. The intra-assay coefficient of variation (CV) was ≤7.8 % for E, ≤2.4 % for NE, while the inter-assay CV was ≤7.9 % for E, ≤2.9 % for NE. For the LCMS assay, the LOQ was 8 pg/mL for E, 15 pg/mL for NE. The intra-assay CV was ≤6.3 % for E, ≤10 % for NE. and the inter-assay CV was ≤6.7 % for E and ≤9.5 % for NE. Due to insufficient serum volume, in 40 sets of paired patient samples (measured by the HPLC-ED method), one or both plasma

specimens (0 and/or 20 min) had to be diluted 1:1 for the E assay, resulting in a LOQ of 40 pg/mL.

The reference range provided by the laboratory, based on the HPLC-ED methodology for adults (no pediatric references were available): supine values, for age 18 years or older is: E 0–58 pg/mL, NE 149–564 pg/mL. For dopamine, the LOQ by the HPLC-ED method was 10 pg/mL, and reference interval for adults was 0–16 pg/mL (supine), 0–27 pg/mL (upright).

Serum copeptin was measured by an automated 2-site immunofluorescent assay (B·R·A·H·M·S Copeptin proAVP KRYPTOR, Thermo-Fisher Scientific) at Quest Laboratories. The KRYPTOR assay is linear from 2 – 500 pmol/L with a LOQ of 2 pmol/L, an intra-assay coefficient of variation (CV) ≤5.2 % and inter-assay CV ≤3.7 %. Reference ranges provided by the Laboratory are 2.4–26 pmol/L for age 3–17.9 years, <13.7 pmol/L for age 18 years or older, with no reference to fluid status. As copeptin levels are affected by water intake in the previous several hours, our own reference values are derived from a series of 85 children, following an overnight fast and fluid restriction of ≥8 h, after exclusion of outliers with very high copeptin levels [8]. In this cohort, the median copeptin concentrations were 8.0 (6, 11.5) pmol/L, with a range of 3–21 pmol/L. For comparison, Tuli et al. [9] reported values (median 10.6 pmol/L, range 3.3–14.9) in children similarly fasted overnight.

The distribution and spread of the data were visually assessed using histograms and boxplots. Data for the analyte concentrations at baseline and 20 min were presented as median (interquartile range) due to non-normal distribution. Significance of the median concentrations between baseline and 20-min samples was assessed via Wilcoxon-Signed Rank test. The shape of the percent changes of the analytes at two time points (i.e., percent change in NE between 0 and 20 min) were assessed to follow a normal distribution. As such, the significance of the percent changes in the mean differences between catecholamines and copeptin was assessed via a one-sample, two-tailed t-test against the reference zero. Correlations between copeptin and catecholamines were determined using Spearman correlation coefficients (r). A simple linear regression model was built using age as the predictor and the delta concentrations as the dependent variables; however, the assumptions of the regression models were significantly violated given non-normal distribution of the delta values. We did not consider transforming the data to conduct the regression analysis due to complexity of the interpretation of output. Significance was determined by a p-value of <0.05. All analyses were completed with IBM SPSS Statistics for Windows, Version 28.0 (Armonk, NY: IBM Corp) and GraphPad Prism version 9.5.1 for Windows (GraphPad Software, Boston, MA).

Results

Blood samples were collected and analyzed from 100 children and adolescents undergoing endocrine testing. Median age was 12 years (range 5–17 years), and 71 % of the participants were male in this cohort.

Norepinephrine (NE) values were available in 99 subjects at both data points (0 and 20 min, paired samples). One sample was missing due to not enough blood quantity. Median (IQR) NE concentration decreased from 349 (244, 482) pg/mL at baseline to 253 (184, 348) pg/mL at 20 min (p<0.001), and this corresponds to an average of 106 pg/mL or a 28 % reduction in mean NE from baseline (p<0.001) (Figure 1A).

Epinephrine (E) values were measured in all 100 subjects at both data points. However, the concentrations were below the detection limit of the assay (20 pg/mL) in 6 subjects; they were <40 pg/mL in another 40 subjects, whose plasma sample had to be diluted 1:1 due to insufficient volume. After these 46 subjects were excluded, paired data were available in 54 subjects. Median (IQR) E changed from 57 (43, 116) pg/mL at baseline to 57 (38, 96) pg/mL at 20 min (p=0.024), and this corresponds to an average of 16 pg/mL or an 18 % reduction in mean E from baseline (p<0.042) (Figure 1B).

Dopamine (DA) values were also measured in all 100 subjects at both data points. However, the concentrations were below the assay's detection limit in 81 subjects (including several subjects whose serum had to be diluted, due to insufficient volume), so paired data were available in only 19 subjects. The median (IQR) DA concentration changed from 21 (19, 26) pg/mL at baseline to 20 (18, 25) pg/mL at 20 min (p=0.083). Mean reduction in percent DA change was also insignificant (p=0.934) (data not shown in the Figure).

Over 95 % of the E, NE and DA values, both at 0 and 20 min, were in the reference range. When the levels measured by the LCMS method (n=14) were converted to HPLC using the appropriate conversion factor, the difference remained significant for NE and E values and insignificant for DA.

Copeptin values were measured in 51 subjects at both data points, but 2 baseline data were missing due to insufficient blood quantity to run the test. As such, paired data was available in 49 subjects. Median (IQR) copeptin concentration changed from 9.4 (6.3, 15.2) pmol/L at baseline to 9 [5, 10] pmol/L at 20 min (p<0.001), and this corresponds to an average of 2.7 pmol/L or a 17 % reduction in mean Copeptin concentration from baseline (p=0.012) (Figure 1C).

We then explored the correlation between percent changes of all variables (NE, E, DA, copeptin) to determine their relationship (Figure 2). We found no correlation

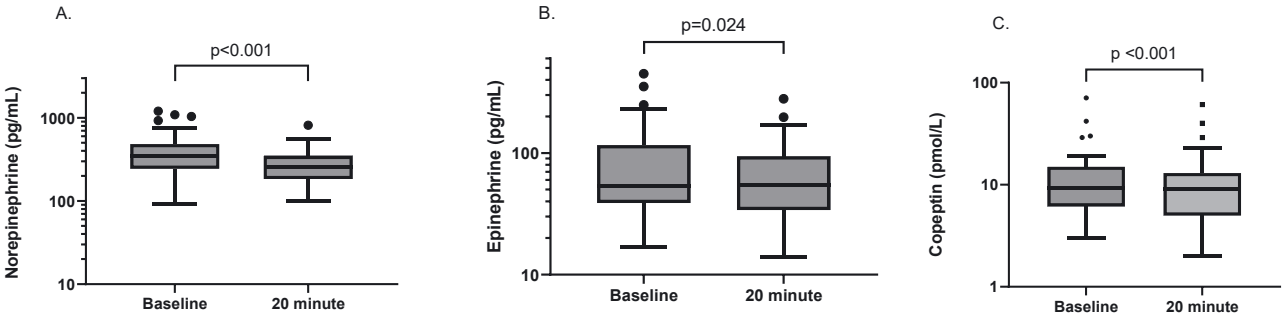

Figure 1: Distribution of norepinephrine, epinephrine and copeptin values at baseline and 20 min. The boxplots indicate the median and IQR, and the whiskers indicate 1.5 times the IQR. Dots represent individual observations outside of the expected range (i.e., outliers).

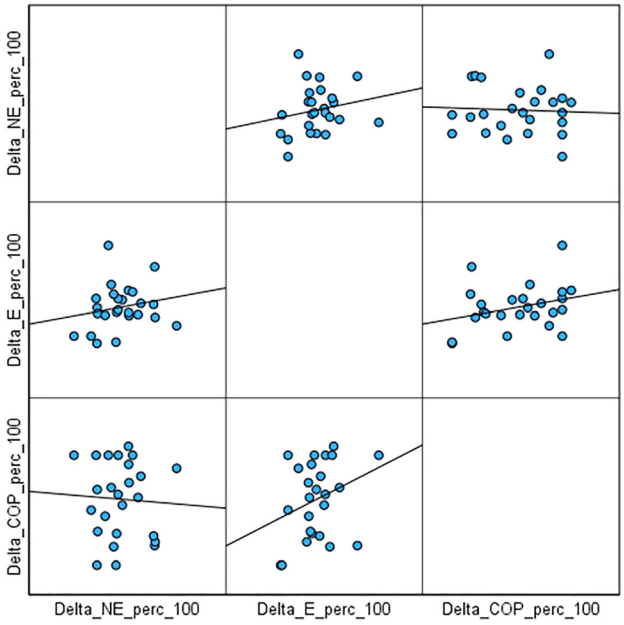

Figure 2: Correlation matrix assessing the linear relationship between percent changes of norepinephrine, epinephrine and copeptin between 0 and 20 min.

between NE and E (n=54; r=0.08, p=0.95), NE and Copeptin (n=48; r=−0.11, p=0.45) or E and Copeptin (n=25, r=0.32, p=0.11) Percent change data for DA vs. the other analytes could not be meaningfully expressed, due to the small sample size. Scatter plot matrix shows the relationship of percent changes of NE, E and copeptin (Figure 2). There was no correlation between age and baseline or +20 min concentrations of E, NE or copeptin.

Discussion

In this study, we measured the plasma catecholamines and serum copeptin levels shortly after insertion of an IV catheter and 20 min later in otherwise healthy children undergoing endocrine stimulation tests as part of their routine care. Our data show that, after 20 min of IVC placement, circulating levels of NE, E, and copeptin decreased significantly.

Venepuncture-associated pain and stress have been the focus of investigation for decades [11, 12], as they have been assumed to falsely increase catecholamine and metanephrine plasma concentrations in subjects being evaluated for pheochromocytomas/paragangliomas (PPGL), resulting in false positive results in unaffected individuals [13]. The study by Eijkelenkamp et al. [10] measured plasma catecholamines and metanephrines in blood samples obtained from an IV cannula in 22 adults, who had been resting for 30 min in the supine position, following the guidelines suggested for the diagnosis of PPGL [14]. They compared those values to the ones obtained immediately after venepuncture in the same subjects from a contralateral vein and found E, but not NE, to be significantly higher after direct venepuncture. They do not specify, however, how long after the venepuncture the blood was obtained. As epinephrine (released by the adrenal glands) and NE (released predominantly by peripheral sympathetic nerves) may take some time to reach the periphery, it is important to standardize the time of blood draw, and it is difficult to compare studies using different experimental conditions.

In our series, we observed a significant decrease in plasma NE and E concentrations from 0 to 20 min, similar to the trend reported by Eichler et al. [11] in a small group of children. Overall, these findings are in keeping with the hypothesis that the stress of IVC induces a rapid increase in NE levels shortly after IV placement, followed by a rapid decrease related to the short half-life of NE once the stimulus of venepuncture abates. Epinephrine plasma concentrations followed the same pattern, and the decrement between baseline and 20 min achieved statistical significance, too, despite a smaller sample size (46 subjects were removed

from data analysis due to undetectable E levels). No trend was noted for dopamine concentrations, but the sample size was too small for a reliable assessment. The different pattern of catecholamine decrease noted with the above-mentioned study in adults [10] may suggest that, in addition to differences in blood sampling methodology, age or other factors may contribute to the variance between studies.

Our study has several weaknesses. We used less-than-ideal experimental conditions. After a few attempts at different positioning of patients, we found that most children were opposed to lying in the supine position at the time of IVC. Thus, for uniformity of sampling conditions, we elected to keep the subjects in the same 45-degree semi-recumbent position, which all the subjects maintained from the beginning to the end of the study. To accommodate our nurses' busy testing schedule, we had our subjects rest in the above noted position for ≥10 min, rather than the ≥20 min generally recommended [15] and we measured the second sample at 20, rather than the 30 min traditionally recommended for catecholamine sampling after venepuncture. The 10 and 20-min intervals we used however, correspond to ~ 3 – 5 half-lives of E and NE [16] and thus should allow sufficient time for clearance of a higher posture-related level or a phlebotomy-related catecholamine "pulse". We acknowledge that prolonging both the initial rest period and the interval between blood draws to 30 min and/or having a control group of children resting for a longer period would have been more definitive, but it would have required much higher financial and personnel resources not available to us for this study in our current clinical setting. Another possible confounder is the known minute-to-minute spontaneous pulsatility of catecholamine levels [17]. As the oscillations of E and NE concentrations occur randomly, it is unlikely that they affected the statistical trend in one direction as we observed. Circadian and ultradian rhythms have not been consistently shown for catecholamines [17], and they would not affect their concentration anyway, within the short interval of 30 min for testing occurring at the same time of the day. Finally, we were unable to measure free plasma metanephrines due to limits on blood volume drawn.

This study also shows, for the first time per our knowledge, a significant decrease in serum copeptin concentrations in the 20-min following phlebotomy, congruent with the decrease in NE and E concentrations during this interval, which suggests a similar stress-related response to the stimulus of IVC for this peptide. We have previously reported that this effect may be amplified and result in extremely elevated copeptin concentrations after venepuncture in a small percentage of children [8] which should be kept in mind while analyzing copeptin concentrations. Lack of correlation between the percent decrease of NE vs. E, NE vs. copeptin, and E vs. copeptin between 0 and 20 min may be related to separate sites of secretion and different regulatory mechanisms for these analytes. Additionally, regarding copeptin vs. catecholamines, the poor correlation could also be related to copeptin having a much longer half-life (estimated to be between 20 and 30 min) [18] than that of catecholamines (a few minutes) in circulation.

It is unclear whether the significant, albeit quantitatively modest, decrement in copeptin concentrations may have practical implications regarding the timing of the blood sample when copeptin is measured for the diagnosis of conditions of water and sodium dysregulation [19]. Regardless, these data suggest that random copeptin concentrations should be interpreted with the knowledge that they could be higher if obtained in the first few minutes of venepuncture rather than several minutes later.

Our conclusions are limited to our specific blood sampling protocol, in which E, NE and copeptin were measured at least 1 min after the venepuncture. If these analytes were to be measured in the first milliliters of blood obtained immediately after the venepuncture, before they achieve systemic concentrations in peripheral blood from their release site, the findings might be different.

This study contributes to the scarce pediatric reference data on plasma E and NE and provides reference values for these hormones and copeptin, that may be useful to objectively assess the response of children to different interventions to limit the pain and stress of intravenous cannulation [20]. It may also contribute to the database on testing used in the work-up for the diagnosis of PPGL in children. At this regard, a limitation of this study is that due to constraints on the volume of blood obtained, we could not measure plasma free metanephrines. Quantitation of these compounds is the preferred methodology for the diagnosis of PPGL due to their plasma concentrations believed to be less variable than those of E and NE, as they are produced continuously and independently of catecholamine release by intracellular O-methylation after leakage of the parent amines from chromaffin granule stores in the cytoplasm [21]. However, the previously quoted study by Eijkelkamp et al. [10] showed that blood sampling by venepuncture resulted in a significantly higher metanephrine, normetanephrine, and epinephrine compared to sampling by an indwelling catheter, suggesting that the same precautions should be used when measuring plasma E, NE, and their meta-derivatives. Reference data on plasma free metanephrines and their response to venepuncture in children, under strict and standardized sampling conditions, are needed to build on the above data.

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Research ethics: The study protocol was approved by the University of Pittsburgh Institutional Review Board (PRO19120068 and PRO23110021). Written informed consent was obtained from the control participants' parent/legal guardian/next of kin prior to participation in the study.

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning Tools: None declared.

Conflict of interest: Dr. Michael J McPhaul is a consultant for Quest Diagnostics Nichols Institute and owns stock in the company. The other authors have no significant conflicts to disclose.

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References

1. Kennedy RM, Luhmann J, Zempsky WT. Clinical implications of unmanaged needle-insertion pain and distress in children. Pediatrics 2008;122:S130–3.
2. Martino M, Arnaldi G. Copeptin and stress. Endocrines 2021;2: 384–404.
3. Robinson AG. The posterior pituitary (Neurohypophysis). In: Gardner DG, Shoback D, editors. Greenspan's basic & clinical endocrinology, 10th ed. New York, NY: McGraw-Hill Education; 2017: 121–36 pp.
4. Mavani GP, DeVita MV, Michelis MF. A review of the nonpressor and nonantidiuretic actions of the hormone vasopressin. Front Med (Lausanne) 2015;2:19.
5. Kluge M, Riedl S, Erhart-Hofmann B, Hartmann J, Waldhauser F. Improved extraction procedure and RIA for determination of arginine8-vasopressin in plasma: role of premeasurement sample treatment and reference values in children. Clin Chem 1999;45: 98–103.
6. Morgenthaler NG, Struck J, Jochberger S, Dunser MW. Copeptin: clinical use of a new biomarker. Trends Endocrinol Metab 2008;19:43–9.
7. Jalleh R, Torpy DJ. The emerging role of copeptin. Clin Biochem Rev 2021;42:17–25.
8. Sastry S, March CA, McPhaul MJ, Garibaldi LR. Very elevated serum copeptin concentrations occur in a subset of healthy children in the minutes after phlebotomy. J Pediatr Endocrinol Metab 2024;37:8–14.
9. Tuli G, Munarin J, Tessaris D, Einaudi S, Matarazzo P, de Sanctis L. Distribution of plasma copeptin levels and influence of obesity in children and adolescents. Eur J Pediatr 2021;180:119–26.
10. Eijkelenkamp K, van Geel EH, Kerstens MN, van Faassen M, Kema IP, Links TP, et al. Blood sampling for metanephrines comparing venipuncture vs. indwelling intravenous cannula in healthy subjects. Clin Chem Lab Med 2020;58:1681–6.
11. Eichler I, Eichler HG, Rotter M, Kyrle PA, Gasic S, Korn A. Plasma concentrations of free and sulfoconjugated dopamine, epinephrine, and norepinephrine in healthy infants and children. Klin Wochenschr 1989;67:672–5.
12. Netter P. Psychological aspects of catecholamine response patterns to pain and mental stress in essential hypertensive patients and controls. J Clin Hypertens 1987;3:727–42.
13. Chortis V, Bancos I, Crowley RK, Arlt W. Supine or sitting plasma metanephrine screening? A unifying solution for patients and doctors. Clin Endocrinol (Oxf) 2015;82:776–7.
14. Darr R, Pamporaki C, Peitzsch M, Miehle K, Prejbisz A, Peczkowska M, et al. Biochemical diagnosis of phaeochromocytoma using plasma-free normetanephrine, metanephrine and methoxytyramine: importance of supine sampling under fasting conditions. Clin Endocrinol (Oxf) 2014; 80:478–86.
15. Pommer G, Pamporaki C, Peitzsch M, Remde H, Deutschbein T, Nolting S, et al. Preanalytical considerations and outpatient versus inpatient tests of plasma metanephrines to diagnose pheochromocytoma. J Clin Endocrinol Metab 2022;107:e3689–e98.
16. FitzGerald GA, Hossmann V, Hamilton CA, Reid JL, Davies DS, Dollery CT. Interindividual variation in kinetics of infused epinephrine. Clin Pharmacol Ther 1979;26:669–75.
17. Schofl C, Becker C, Prank K, von zur Muhlen A, Brabant G. Twenty-four-hour rhythms of plasma catecholamines and their relation to cardiovascular parameters in healthy young men. Eur J Endocrinol 1997;137:675–83.
18. Fenske WK, Schnyder I, Koch G, Walti C, Pfister M, Kopp P, et al. Release and decay kinetics of copeptin vs AVP in response to osmotic alterations in healthy volunteers. J Clin Endocrinol Metab 2018;103:505–13.
19. Bonnet L, Marquant E, Fromonot J, Hamouda I, Berbis J, Godefroy A, et al. Copeptin assays in children for the differential diagnosis of polyuria-polydipsia syndrome and reference levels in hospitalized children. Clin Endocrinol (Oxf) 2022;96:47–53.
20. Cozzi G, Valerio P, Kennedy R. A narrative review with practical advice on how to decrease pain and distress during venepuncture and peripheral intravenous cannulation. Acta Paediatr 2021;110:423–32.
21. Havekes B, van der Klaauw AA, Weiss MM, Jansen JC, van der Mey AG, Vriends AH, et al. Pheochromocytomas and extra-adrenal paragangliomas detected by screening in patients with SDHD-associated head-and-neck paragangliomas. Endocr Relat Cancer 2009; 16:527–36.