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Original Article



ORIGINAL ARTICLE

# Plasma copeptin is increased and associated with smaller kidney volume in young adults born very preterm

Adrien Flahault[1], Guillaume Bollée[2], Ramy El-Jalbout[1,3], Anik Cloutier[1], Robson A.S. Santos[4], Anne-Laure Lapeyraque[1,5], Thuy Mai Luu[1,6] and Anne Monique Nuyt[1,7]

[1]Sainte-Justine University Hospital Research Center, Université de Montréal, Montréal, Québec, Canada, [2]Division of Nephrology, Centre Hospitalier de l'Université de Montréal, Université de Montréal, Montréal, Québec, Canada, [3]Medical Imaging Department, Sainte-Justine University Hospital, Université de Montréal, Montréal, Québec, Canada, [4]Department of Physiology and Biophysics, National Institute of Science and Technology in Nanobiopharmaceutics (INCT-Nanobiofar), Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [5]Division of Nephrology, Department of Pediatrics, Sainte-Justine University Hospital, Université de Montréal, Montréal, Québec, Canada, [6]Division of General Pediatrics, Department of Pediatrics, Sainte-Justine University Hospital, Université de Montréal, Montréal, Québec, Canada and [7]Division of Neonatology, Department of Pediatrics, Sainte-Justine University Hospital, Université de Montréal, Montréal, Québec, Canada

Correspondence to: Anne Monique Nuyt and Thuy Mai Luu; E-mail: anne.monique.nuyt@umontreal.ca and thuy.mai.luu@umontreal.ca

## ABSTRACT

**Background.** Plasma copeptin, a surrogate marker for vasopressin levels, is increased in neonates born preterm, particularly in those with a more severe neonatal course, as reflected by bronchopulmonary dysplasia. Copeptin levels in adulthood are unknown.

**Methods.** In this case–control study of 101 adults born very preterm (<30 weeks of gestation) and 105 control adults born full-term, a comprehensive clinical and biological assessment was performed, including blood pressure measurements, kidney ultrasound and determination of plasma copeptin, renin activity, angiotensin II, aldosterone, apelin, sodium and potassium, serum and morning urine osmolality.

**Results.** The median age in the study was 23.1 years [interquartile range (IQR) 21.2–24.8] and 57% were females. In males, the median copeptin levels were 8.2 pmol/L (IQR 6.3–12.4) and 6.1 pmol/L (IQR 4.3–9.0) in the preterm and term groups, respectively ($P = 0.022$). In females, the median copeptin levels were 5.2 pmol/L (IQR 3.9–7.6) and 4.0 pmol/L (IQR 2.8–5.7) in the preterm and term groups, respectively ($P = 0.005$). Adults born preterm with a history of bronchopulmonary dysplasia had further increased copeptin levels. The kidney volume, adjusted for height, was smaller and albuminuria was higher in the preterm group, and both were associated with higher plasma copeptin levels.

**Conclusions.** Plasma copeptin is higher in young adults born preterm and is related to a more severe neonatal course and smaller kidney volume.

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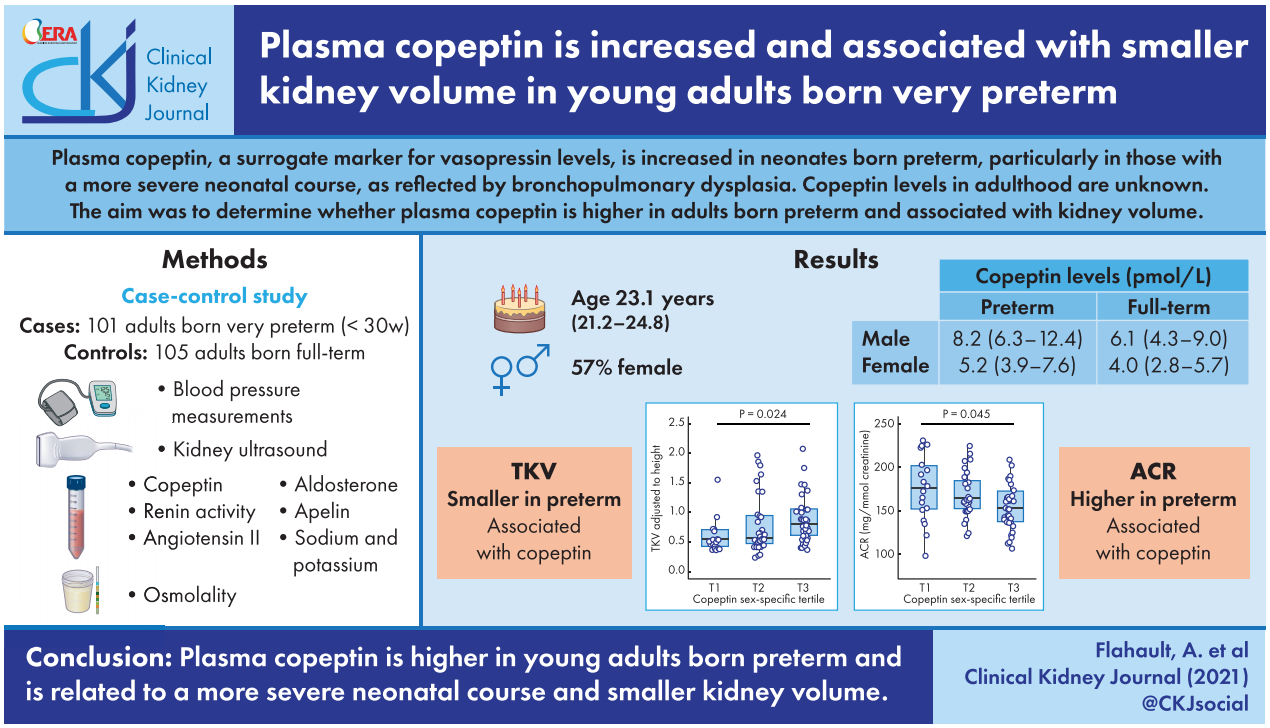
## GRAPHICAL ABSTRACT




**Keywords:** aldosterone, apelin, kidney function, neonatalogy, renin

## INTRODUCTION

Around 1% of births occur at <30 weeks gestational age (GA) [1] and the majority survive to adulthood [2]. Preterm birth occurs during a critical period of nephrogenesis and is associated with lower nephron endowment and smaller kidney volume [3]. To maintain the glomerular filtration rate (GFR) with a lower nephron number, glomerular hyperfiltration at the single-nephron level must occur. However, over the long term, this may damage the kidney and increase the risk of renal disease [4]. Individuals born preterm are at increased risk of chronic kidney disease (CKD) and kidney failure and have higher albumin excretion rates [5–8]. Most studies on long-term kidney function in preterm children and adults do not report a significant impact on blood creatinine or estimated or measured glomerular filtration rate (eGFR or mGFR) [9–11]. Kidney histological studies have suggested that preterm neonates show signs of single-nephron glomerular hyperfiltration [12], and authors have reported a higher eGFR or mGFR per unit of kidney volume in their cohorts of preterm children [13] or adults [11], suggestive of the single-nephron glomerular hyperfiltration.

Mechanistic pathways leading to glomerular hyperfiltration have not been determined in those born preterm. The components of the renin–angiotensin–aldosterone system (RAAS), arginine vasopressin (AVP) [14] and apelin, a peptide that is regulated in opposition to AVP and counteracts its antidiuretic effects [15–18], all exert haemodynamic effects on afferent and efferent glomerular arterioles. These may play a role in increasing renal blood flow to the glomerulus, which modulates single-nephron glomerular filtration. However, circulating renin or plasma renin activity (PRA), angiotensin II (Ang II) and aldosterone levels are similar in adolescents [19] and adults [8] born preterm versus term, suggesting no increase in the stimulation of RAAS.

The studies conducted on preterm neonates have shown increased AVP levels as well as an increase in plasma levels of copeptin, a surrogate marker of AVP levels [20], and especially so in those who develop bronchopulmonary dysplasia (BPD), the most frequent complication of preterm birth [21–25] and a marker of a more severe neonatal course. To our knowledge, copeptin and apelin levels have not previously been measured in adults born preterm. In addition, possible associations of copeptin and apelin with kidney volume and albuminuria remain largely undocumented.

We hypothesized that plasma copeptin levels are higher in adults born preterm and are associated with a more severe neonatal course.

## MATERIALS AND METHODS

### Study population and design

The results presented in this research were obtained from the Health of Adults born Preterm Investigation (HAPI) participants. The study population and design have previously been reported [26, 27]. Briefly, 101 adults ages 18–29 years born preterm before 30 weeks gestational age (GA) and 105 full-term controls (GA ≥37 weeks) of the same age, recruited among friends and siblings, were included in the study. The participants with severe neurocognitive impairment and pregnancy were excluded. Approval was granted by the Sainte-Justine University Hospital, McGill University Health Centre and Sir Mortimer B. Davis Jewish

General Hospital Research Ethics Boards. All participants gave written informed consent to participate in the study.

### Clinical and radiological assessments

Kidney ultrasonography was performed by the trained radiology technicians and interpreted by a staff radiologist (R.E.J.), as previously described [3]. The ultrasound images were obtained while the participant was lying in the supine position or in an oblique position, depending on the side where measurements were taken. The curvilinear ultrasound probe was placed in a longitudinal position from the anterior lateral position and the longest axis of the kidney was measured. Then the probe was turned to 90° at the center of the kidney and the laterolateral and anteroposterior axes were measured at the level of the renal hilum. The kidney volume was calculated using the equation length × width × depth (anterior–posterior diameter) × $\pi/6$ [6]. Further information regarding the clinical assessments and definition of BPD are provided in the Supplementary file.

### Biological measurements

Blood and urine samples were collected after measuring the blood pressure in the early morning, after 12 h of fasting. Using this first blood sample, the plasma creatinine was used to estimate the glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [28]. The plasma copeptin and apelin levels were also determined from this first sample. The second blood sample was used for determining PRA, Ang II and aldosterone levels, which was collected after patients had been resting in a supine position for 30 min. All measurements were performed according to the manufacturer's recommendations. Copeptin determinations and clinical biochemistry were performed in singlicate, where the intra-assay coefficient of variation (CV) was low. All other determinations were performed in duplicates. Determinations in which CV between duplicates was above the manufacturer's specifications were repeated.

Specific methods for the biological measurements used in this research are provided in the Supplementary file.

### Statistical analysis

Results are shown as medians [interquartile range (IQR)] or *n* (%). Unadjusted comparisons between the groups were performed using the Mann–Whitney U test (continuous variables, two groups), the Kruskal–Wallis test (continuous variables, >2 groups, followed by Dunn's test with Holm's adjustment) or the Fisher exact test (categorical variables) independent of the distribution of data. Correlation coefficients and P-values were calculated using the Spearman method. For adjusted comparisons, the associations between variables were assessed using multivariate linear models for variables with normal or log-normal distribution. We obtained estimates, 95% confidence intervals (CIs) and P-values. P-values <0.05 were considered statistically significant. All analyses were performed using R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) [29]. Normality of residuals was assessed visually using the Shapiro–Wilk test. Plasma copeptin level distribution was not normal and log-transformed plasma copeptin followed an approximate normal distribution, but the Shapiro–Wilk test remained significant ($P < 0.001$). Plasma copeptin values are known to be higher in males than females [30, 31]. In order to assess the association of sex-adjusted plasma copeptin levels with the variables of interest, we compared values among sex-specific tertiles of copeptin using the Kendall test (continuous variables) or the Cochran–Armitage test (categorical variables). Sex-specific tertiles were obtained from each group and increasing plasma copeptin ranges for each sex-specific tertile are provided in Table 3 and Supplementary data, Table S4. This statistical approach has been previously used in epidemiological studies from different groups that evaluate the association of plasma copeptin with other variables of interest, where the distribution of copeptin was non-normal [30–34]. For consistency in the analysis, we also studied the association of plasma apelin, renin activity, Ang II and aldosterone with the covariates of interest in sex-specific tertiles. For plasma copeptin, we also provide P-values obtained using non-parametric kernel regression [35]. Our study had an 80% power to identify a difference of 0.4 standard deviation between the two groups with an alpha error of 5%.

### Missing data

The number of missing data is provided in the tables. The measure of PRA, angiotensin peptides and aldosterone was an amendment to the initial study protocol, therefore measures were not obtained for all the study subjects. The data for the biological measurement were missing due to the absence of the sample.

## RESULTS

### Study population

Study participant characteristics are provided in Table 1. Median GA at birth was 27.4 weeks (IQR 26–28) for the preterm and 40 weeks (IQR 39–40) for the term subjects. Thirty-seven (37%) of the participants born preterm had a more severe neonatal course, as shown by a history of moderate–severe BPD. The median age of participants on the day of the study was 23 years.

### Kidney function

The preterm participants were smaller than full-term participants. The serum creatinine levels and eGFRs were similar between the groups. Total kidney volume (TKV) was smaller even after adjustment for height or body surface area (BSA). As previously reported in a large subset of participants [8], higher urinary albumin:creatinine ratio (ACR) was higher in the preterm group (Table 1).

### Dietary intake

Term and preterm participants reported similar daily dietary intake of sodium, potassium, phosphorus, calcium, magnesium, glucose, proteins, lipids, calories and daily water consumption (Supplementary data, Table S1).

### Plasma copeptin is increased in young adults born preterm

PRA plasma Ang II (previously reported [8]), plasma aldosterone, aldosterone:PRA ratio and plasma apelin levels were similar between the groups. In contrast, plasma copeptin levels were higher in the preterm group, both in males and females (Table 2).

**Table 1. Study participant characteristics**

| Characteristics | Term ($n = 105$) | | Preterm ($n = 101$) | | P-value |
|---|---|---|---|---|---|
| | Missing, *n* (%) | Median (IQR) or *n* (%) | Missing, *n* (%) | Median (IQR) or *n* (%) | |
| Neonatal characteristics | | | | | |
| Male | 0 (0) | 43 (41) | 0 (0) | 45 (45) | 0.67 |
| White ethnicity | 0 (0) | 95 (90) | 0 (0) | 91 (90) | >0.99 |
| Gestational age (weeks) | 1 (1) | 40 (39–40.3) | 0 (0) | 27.4 (26.1–28.2) | – |
| Birthweight (g) | 0 (0) | 3400 (3140–3670) | 0 (0) | 920 (795–1125) | – |
| Small for gestational age | 1 (1) | 7 (7) | 0 (0) | 6 (6) | >0.99 |
| Pregnancy-induced hypertension | 0 (0) | 11 (10) | 0 (0) | 29 (29) | 0.001 |
| Moderate–severe BPD | – | – | 1 (1) | 37 (37) | – |
| Adult characteristics on study day | | | | | |
| Age (years) | 0 (0) | 23.1 (21.3–24.8) | 0 (0) | 23.0 (21.2–24.8) | 0.97 |
| Height (cm) | 0 (0) | 170 (164–176) | 0 (0) | 165 (159–171) | <0.001 |
| Weight (kg) | 0 (0) | 65.1 (59.4–77) | 0 (0) | 60.5 (51.9–69.5) | <0.001 |
| Body mass index | 0 (0) | 22.4 (20.5–25.5) | 0 (0) | 21.8 (19.9–24.7) | 0.078 |
| Body surface area ($m^2$) | 0 (0) | 1.76 (1.66–1.94) | 0 (0) | 1.68 (1.54–1.8) | <0.001 |
| Clinical characteristics | | | | | |
| Total kidney volume ($cm^3$) | 18 (17) | 320 (275–375) | 12 (12) | 263 (232–301) | <0.001 |
| Total kidney volume corrected for height ($cm^3/m$) | 18 (17) | 193 (160–211) | 12 (12) | 162 (139–183) | <0.001 |
| Total kidney volume corrected for BSA ($cm^3/m^2$) | 18 (17) | 178 (156–200) | 12 (12) | 162 (137–177) | <0.001 |
| Plasma creatinine (μmol/L) | 2 (2) | 68 (63–75) | 5 (5) | 68.5 (62–76) | 0.93 |
| eGFR (CKD-EPI) (mL/min/1.73 $m^2$) | 2 (2) | 119 (113–124) | 5 (5) | 122 (112–125) | 0.27 |
| ACR (mg/mmol) | 2 (2) | 0.625 (0.45–0.818) | 2 (2) | 0.694 (0.509–1.06) | 0.023 |
| SBP (mmHg) | 1 (1) | 118 (111–125) | 4 (4) | 123 (115–130) | 0.005 |
| DBP (mmHg) | 1 (1) | 71 (66–75) | 4 (4) | 73 (68–77) | 0.022 |
| Pulse pressure (mmHg) | 1 (1) | 47.5 (43.8–52) | 4 (4) | 49.5 (43–54) | 0.13 |
| Use of antihypertensive medication | 0 (0) | 2 (2) | 0 (0) | 1 (1) | 1 |
| Fasting glucose (mmol/L) | 2 (2) | 4.8 (4.5–5) | 5 (5) | 4.8 (4.5–5.1) | 0.31 |
| Use of antidiabetic medication | 0 (0) | 1 (1) | 0 (0) | 1 (1) | 1 |

SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate using the CKD-EPI equation. P-values were calculated using the Mann–Whitney U test or the Fisher's exact test.

**Table 2. Biomarkers of kidney haemodynamics and water/sodium homeostasis and plasma and urine electrolytes and osmolality**

| Biomarkers | Term ($n = 105$) | | Preterm ($n = 101$) | | P-value |
|---|---|---|---|---|---|
| | Missing, *n* (%) | Median (IQR) | Missing, *n* (%) | Median (IQR) | |
| Renovascular biomarkers | | | | | |
| Plasma renin activity (ng/mL/h) | 26 (25) | 1.32 (0.71–2.2) | 26 (26) | 1.42 (0.74–2.21) | 0.64 |
| Plasma angiotensin II (pg/mL) | 40 (38) | 2.67 (2.22–3.50) | 42 (42) | 3.00 (2.17–3.50) | 0.39 |
| Plasma aldosterone (pg/mL) | 26 (25) | 64.5 (49.2–87.3) | 26 (26) | 66.7 (51.2–89.7) | 0.80 |
| Aldosterone:plasma renin activity ratio | 26 (25) | 48.8 (34–81) | 26 (26) | 50.2 (29.7–80.3) | 0.70 |
| Copeptin (pmol/L) | | | | | |
| Copeptin (males) (pmol/L) | 3 (3) | 6.05 (4.30–8.95) | 8 (8) | 8.20 (6.25–12.4) | 0.022 |
| Copeptin (females) (pmol/L) | | 3.95 (2.75–5.65) | | 5.15 (3.90–7.55) | 0.005 |
| Apelin (ng/mL) | 7 (7) | 0.652 (0.533–0.792) | 10 (10) | 0.74 (0.545–0.861) | 0.09 |
| Blood and urine electrolyte balance | | | | | |
| Serum osmolality (mOsm/kg $H_2O$) | 3 (3) | 291 (287–294) | 9 (9) | 292 (288–297) | 0.12 |
| Plasma sodium (mmol/L) | 2 (2) | 139 (137–140) | 4 (4) | 139 (138–140) | 0.17 |
| Plasma potassium (mmol/L) | 2 (2) | 3.9 (3.8–4.1) | 7 (7) | 3.9 (3.8–4.1) | 0.89 |
| Morning fasting urine osmolality (mOsm/kg $H_2O$) | | | | | |
| Urine osmolality (males) | 1 (1) | 879 (592–1035) | 1 (1) | 873 (780–962) | 0.95 |
| Urine osmolality (females) | | 749 (527–935) | | 869 (693–964) | 0.11 |

Osmolality was measured in plasma and urine. Copeptin T1, T2 and T3 correspond to the first, second and third copeptin sex-specific tertiles. P-values are calculated using the Mann–Whitney U test.

Plasma copeptin levels were significantly higher in males, independent of term/preterm status (Supplementary data, Table S2). Plasma sodium and potassium as well as measured plasma and morning fasting urine osmolality were similar between the groups (Table 2).

## Plasma copeptin is associated with serum and urine osmolality irrespective of term/preterm status

Higher plasma sodium, serum and urine osmolality levels were associated with higher plasma copeptin levels (Figure 1), and

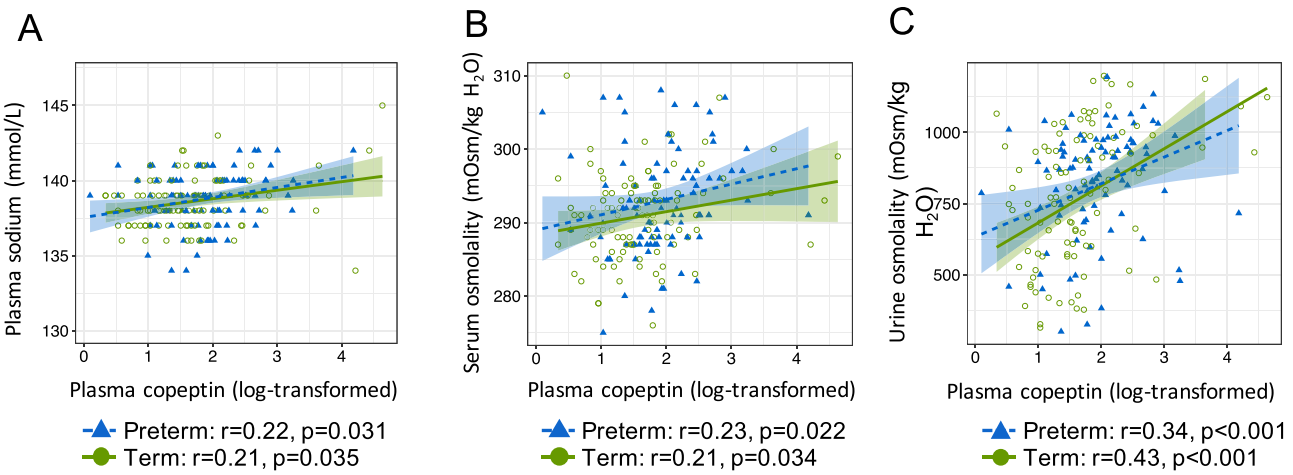



**FIGURE 1:** Plasma copeptin levels (pmol/L, log-transformed) according to serum and urine osmolality in the term and preterm groups. Each figure displays individual determinations of (**A**) plasma sodium, (**B**) measured serum osmolality and (**C**) measured morning fasting osmolality in participants born term (open green circles) or preterm (full blue triangles), according to log-transformed plasma copeptin levels. A linear regression line for the association between the two variables with its computed 95% CI (shaded area) is also provided (dashed blue line, preterm group; full green line, term group). Correlation coefficients (*r*) and P-values were calculated using the Spearman method.

preterm birth did not have a significant effect on this association (Supplementary data, Table S3). Only 5 (5%) term and 2 (2%) preterm participants had plasma copeptin levels higher than expected for serum osmolality. Plasma copeptin levels were not associated with PRA, aldosterone or apelin levels, neither in the preterm group (Table 3) nor in the term group (Supplementary data, Table S4).

### Association of plasma copeptin with severity of neonatal course

When stratifying the preterm group according to history of BPD, a marker of a severe neonatal course (Figure 2A), we found that those with moderate–severe BPD had the highest plasma copeptin levels [median 8.9 pmol/L (IQR 6.5–12.1)], significantly higher than in those with mild or no BPD [5.9 pmol/L (IQR 3.9–8.3); $P = 0.010$] and to those born full-term [4.75 pmol/L (IQR 3.05–6.65); $P < 0.001$]. Plasma copeptin levels were also significantly higher in those born preterm with mild or no BPD than in those born full-term ($P = 0.045$). These results were consistent with those obtained with sex-specific tertiles. Indeed, a history of moderate–severe BPD and other markers of severity of neonatal disease, including older age at continuous positive airway pressure (CPAP)/mechanical ventilation and oxygen weaning, were associated with higher sex-specific plasma copeptin level tertiles. In contrast, gestational age, birthweight percentile, APGAR score at birth, use of non-steroidal anti-inflammatory drugs and neonatal sepsis were not significantly associated with plasma copeptin levels (Table 3). PRA, plasma Ang II, plasma aldosterone and plasma apelin levels did not differ according the history of BPD (Table 4). In a multivariate analysis, male sex and history of moderate–severe BPD were associated with higher plasma copeptin levels, independent of gestational age and birthweight percentile (Table 5).

### Association of plasma copeptin with albuminuria and kidney volume

In the preterm group, higher plasma copeptin levels were associated with higher ACR and smaller kidney volume corrected for height (Figure 2B–D). Among those born preterm, individuals with a history of BPD had a higher ACR and a trend towards smaller kidney volume corrected for height.

### Sensitivity analyses

P-values obtained using kernel non-parametric multivariate regression were similar to those obtained with multivariate linear regression, showing validity of the models used in Supplementary data, Tables S2 and S3.

## DISCUSSION

In this study we showed that preterm birth was associated with increased copeptin levels, a surrogate marker of plasma AVP levels, in adulthood. We found no difference in plasma osmolality between the groups, and the kidney sensitivity to AVP appeared unaltered. We did not find significant differences in the circulating levels of the other vasoactive compounds. In addition, we did not observe any association between copeptin levels and apelin, PRA, Ang II and aldosterone, suggesting the increase in AVP levels is not secondary to RAAS activation or to apelin stimulation. We further showed that a more severe neonatal course was associated with a more important elevation of copeptin levels in preterm adults. Finally, higher plasma copeptin was associated with higher ACR and lower kidney volume in those born preterm.

Population-based studies have shown a higher risk of CKD and kidney failure in individuals born preterm [5, 7]. More than 60% of nephrons are formed during the last trimester. Preterm birth halts this process, leading to lower nephron endowment [3]. Histological analyses of kidney tissues from deceased preterm neonates or biopsies in children reveal a lower glomerular density but an increased glomerular volume when compared with the term controls [12, 36–38]. At 2 years of age, children born preterm have a smaller TKV but a similar eGFR [13]. In our study, we also found a smaller TKV, after adjustment for height, in the preterm group, while eGFR was similar between the groups. These results are in line with a previous study

**Table 3. Levels of biomarkers of kidney haemodynamics, neonatal characteristics and blood pressure according to plasma copeptin sex-specific tertiles in the preterm group**

| | Copeptin sex-specific tertile | | | |
|---|---|---|---|---|
| Characteristics | T1 (pmol/L) ($n = 32$) females (1.7–4.1) males (1.1–6.6) Median (IQR) or *n* (%) | T2 (pmol/L) ($n = 30$) females (4.1–7.0) males (6.6–11.7) Median (IQR) or *n* (%) | T3 (pmol/L) ($n = 31$) females (7.0–65.6) males (11.7–25.8) Median (IQR) or *n* (%) | P-value (trend) |
| Reno-vascular biomarkers | | | | |
| Plasma renin activity (ng/mL/h) | 1.42 (0.86–2.03) | 1.68 (0.88–1.93) | 1.08 (0.63–2.51) | 0.82 |
| Plasma angiotensin II (pg/mL) | 3 (2.46–3.5) | 3.08 (2.71–4.37) | 2.74 (1.52–3.75) | 0.55 |
| Plasma aldosterone (pg/mL) | 61.7 (50.3–74.6) | 70.1 (53.4–89) | 69.7 (55.6–92.2) | 0.27 |
| Plasma apelin (ng/mL) | 0.756 (0.528–0.867) | 0.73 (0.598–0.869) | 0.74 (0.593–0.849) | 0.97 |
| Neonatal characteristics | | | | |
| Gestational age (weeks) | 27.7 (26.6–28.4) | 27.3 (26.1–27.9) | 27.4 (25.6–28.4) | 0.17 |
| Birthweight (g) | 1013 (875–1133) | 930 (823–1096) | 885 (745–1170) | 0.16 |
| Birthweight percentile | 31 (20–46) | 43 (26–53) | 34 (21–51) | 0.53 |
| APGAR score (1 min) | 7 (3–8) | 5 (3–6) | 5 (3–8) | 0.38 |
| APGAR score (5 min) | 8 (7–9) | 7 (5–8) | 7 (6–9) | 0.22 |
| Moderate–severe BPD | 4 (13) | 11 (37) | 16 (52) | <0.001 |
| Age of CPAP/mechanical ventilation weaning (days of life) | 14 (1.5–28) | 31.5 (4.3–40) | 29 (7.8–51.8) | 0.028 |
| Age of oxygen weaning (days of life) | 29.5 (2.8–52.5) | 57 (16–74) | 70 (31–97) | 0.004 |
| Indometacin use | 9 (31) | 14 (48) | 15 (54) | 0.086 |
| Neonatal sepsis | 2 (6) | 6 (21) | 5 (16) | 0.26 |
| Blood pressure and kidney function | | | | |
| SBP (mmHg) | 130 (119–136) | 122 (114–128) | 123 (111–128) | 0.009 |
| DBP (mmHg) | 74 (72–78) | 73 (67–76) | 71 (67–77) | 0.148 |
| Pulse pressure (mmHg) | 52 (48–57.8) | 49.5 (45.5–54) | 47.5 (42–52) | 0.013 |
| eGFR (CKD-EPI) (mL/min/1.73 $m^2$) | 122 (114–125) | 122 (107–124) | 121 (114–125) | 0.57 |
| Fasting glucose (mmol/L) | 5 (5–5) | 5 (5–5) | 5 (5–5) | 0.56 |
| Serum osmolality (mOsm/kg $H_2O$) | 292 (287–298) | 291 (287–295) | 293 (291–299) | 0.19 |
| Morning fasting urine osmolality (mOsm/kg $H_2O$) | 806 (555–897) | 890 (783–948) | 955 (803–1029) | <0.001 |

SBP: systolic blood pressure; DBP: diastolic blood pressure. P-values were calculated using the Kendall or Cochran–Armitage test.

conducted among adults born preterm [11] and are suggestive of glomerular hyperfiltration.

Single-nephron hyperfiltration thus allows maintenance of GFR within the normal range in adults born preterm. The principal mechanistic pathways considered to be activated in hyperfiltration include an imbalance in vasoactive factors controlling pre- and post-glomerular arteriolar tone [39]. This adaptive mechanism accelerates glomerulosclerosis and nephron loss [40] and damages the endothelial/glomerular barrier, increasing albuminuria [4, 41]. Thus young adults born preterm with hyperfiltration may be more prone to kidney disease later in life.

We found that participants born preterm with the highest level of plasma copeptin were the ones with the smallest kidneys and the highest ACR, suggesting that copeptin levels are associated with glomerular hyperfiltration. This hypothesis is strengthened by the fact that the experimental studies suggest that AVP participates in glomerular hyperfiltration [42] and that AVP contributes in kidney disease by its action on the AVP type 2 receptors (V2R) [43]. High plasma copeptin levels have been shown to predict, before the clinical manifestations of disease, the occurrence of pre-eclampsia [44], CKD [30], type 2 diabetes [45] and coronary artery disease [46], which are all more frequent in individuals born preterm [7, 47–49]. Yeung *et al.* [44] report baseline mean plasma copeptin levels of 3.8 pmol/L in women 20 years of age who do not develop pre-eclampsia and 5.1 pmol/L in those who develop pre-eclampsia. These differences are close to those observed in females born preterm versus term in our cohort, suggesting that the differences in plasma copeptin levels observed in our study are clinically significant.

It is possible that AVP exerts a causal role in the development of these diseases. Indeed, in rodents, chronic infusion of AVP replicates the clinical features of pre-eclampsia [50], and a chronic infusion of V2R agonist desmopressin induces kidney disease [51]. In contrast, experimental data suggest that V2R antagonists may exert a protective role in a rodent model of pre-eclampsia [50]. Thus an increase in AVP levels in the young adults born preterm may contribute to their increased burden of chronic diseases. As this study provides evidence of upregulated AVP levels (using copeptin as a surrogate marker) in adults born preterm, it may thus provide insights into the pathophysiology of diseases including CKD and pre-eclampsia in this population [30, 44].

In our study, we also demonstrated that a more severe neonatal course, as shown by a history of moderate–severe BPD, was associated with a further increase in plasma copeptin levels in early adulthood and with higher ACR than in those born preterm with a less severe neonatal course. These results are in line with several reports that had previously associated increased AVP levels with BPD in neonates [21–23]. These results are suggestive of long-term dysregulation of AVP levels following very preterm birth, especially in cases of severe neonatal disease, with potentially adverse consequences on kidney function. Although our study design does not allow us to conclude a causal relationship between a history of moderate–severe BPD and higher plasma

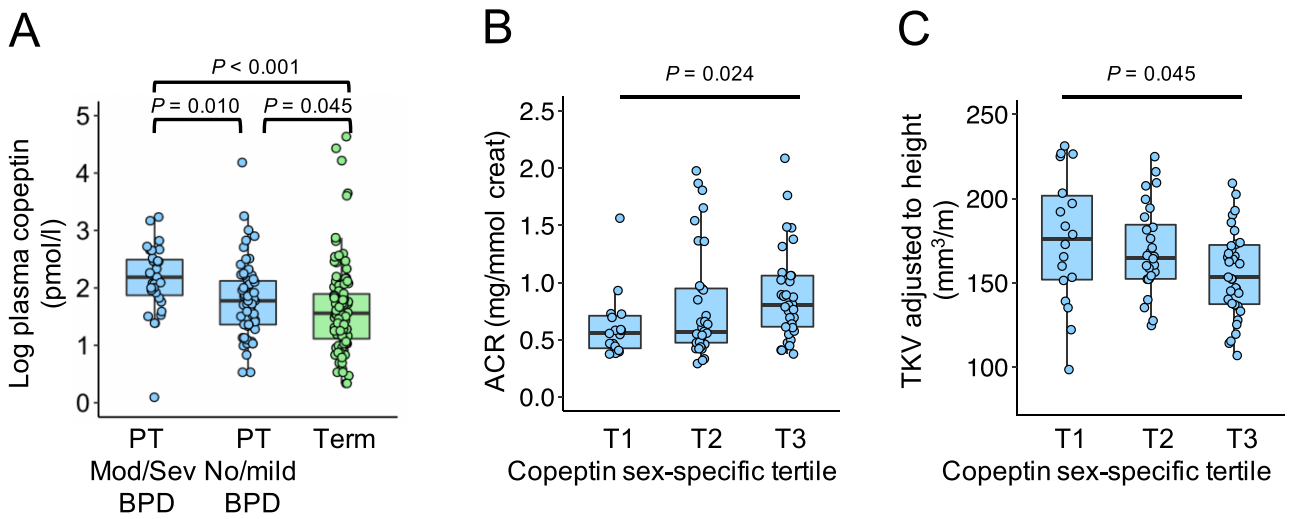



**FIGURE 2:** Association of copeptin levels with neonatal and clinical characteristics in adults born preterm. Each figure displays individual values (rounds) in preterm (PT, light blue) and term (**A**: only, light green) participants. (**A**) Log-plasma copeptin values according to the term/preterm status and history of moderate–severe BPD, defined by respiratory support at 36 weeks post-menstrual age. P-value for Kruskal–Wallis test was <0.001. P-values shown are from post hoc comparisons using Dunn's test. (**B**) Urinary ACR according to sex-specific copeptin tertile. (**C**) TKV adjusted to body height according to sex-specific copeptin tertile. P-values for (B) and (C) were calculated using the Kendall test.

**Table 4. Biomarkers and clinical parameters in adults born preterm according to history of BPD**

| | No BPD ($n = 63$) | | BPD ($n = 37$) | | |
|---|---|---|---|---|---|
| Characteristics | Missing, *n* (%) | Median (IQR) or *n* (%) | Missing, *n* (%) | Median (IQR) or *n* (%) | P-value |
| Reno vascular biomarkers | | | | | |
| Plasma renin activity (ng/mL/h) | 9 (14) | 1.39 (0.77–2.14) | 17 (46) | 1.71 (0.65–2.03) | 0.81 |
| Plasma angiotensin II (pg/mL) | 22 (35) | 2.83 (2.33–3.5) | 20 (54) | 3.17 (2.17–4.5) | 0.82 |
| Plasma aldosterone (pg/mL) | 9 (14) | 68.8 (52.7–92.5) | 17 (46) | 58.5 (50–87.8) | 0.51 |
| Copeptin (pmol/L) | 2 (3) | 5.9 (3.9–8.2) | 6 (16) | 8.9 (6.5–12.1) | 0.001 |
| Apelin (ng/mL) | 4 (6) | 0.756 (0.565–0.849) | 6 (16) | 0.705 (0.536–0.879) | 0.78 |
| Neonatal characteristics | | | | | |
| Gestational age (weeks) | 0 (0) | 27.6 (26.1–28.4) | 0 (0) | 27.3 (26.2–27.9) | 0.115 |
| Birthweight (g) | 0 (0) | 1000 (875–1170) | 0 (0) | 860 (720–950) | 0.002 |
| Birthweight percentile | 0 (0) | 35 (25–52) | 0 (0) | 29 (14–49) | 0.057 |
| Indometacin use | 6 (10) | 14 (25) | 1 (3) | 28 (78) | <0.001 |
| Neonatal sepsis | 0 (0) | 9 (14) | 1 (3) | 6 (17) | 0.78 |
| Clinical characteristics | | | | | |
| ACR (mg/mmol) | 1 (2) | 0.586 (0.451–0.934) | 1 (3) | 0.877 (0.629–1.577) | 0.002 |
| TKV corrected for height ($cm^3/m$) | 7 (11) | 165 (151–192) | 5 (14) | 152 (135–173) | 0.064 |

P-values are calculated using the Mann–Whitney U test.

copeptin levels in young adulthood, we found that the association of BPD and plasma copeptin was independent of sex, gestational age and birthweight percentile.

Our study has several limitations. First, we used creatinine-based equations to estimate GFR rather than measurement, which may be less precise, especially for normal values [52]. However, our results are in line with the literature on the subject, including one study with measured GFR that found similar results [11]. Second, we used sonographic assessments to estimate kidney volume, which may be less precise than magnetic resonance imaging. Again, our results agree with the majority of the previous reports, both in children and in adults. Third, these results were obtained from a mostly white population born in the province of Quebec, Canada. Additional studies on different cohorts will be required to generalize our findings to other preterm populations. Last, we did not measure AVP levels directly but used copeptin as a surrogate marker. Copeptin has been found to strongly correlate to plasma osmolality following osmotic challenge rather than AVP measured by RIA ($r = 0.77$ and 0.49, respectively) [53]. Copeptin has also been shown to be accurate for the diagnosis of diabetes insipidus, a condition where AVP secretion or sensitivity of the kidney to AVP is impaired [54]. Copeptin is therefore a good surrogate for AVP levels in humans. In our study, we observed a significant correlation between urine osmolality and plasma copeptin and between plasma osmolality and plasma copeptin. The association between urine or plasma osmolality and copeptin was similar in the term and preterm groups, suggesting that plasma copeptin is a reliable marker of the effect of AVP on water balance.

Our study also has strengths. It is the first, to our knowledge, to have measured plasma copeptin and apelin levels in a

**Table 5. Estimated effects of sex, gestational age, birth weight percentile and BPD on plasma copeptin levels**

| | Log copeptin (pmol/L) | |
|---|---|---|
| | Estimate (95% CI) | P-value |
| Male sex | 0.37 (0.11–0.63) | 0.006 |
| Gestational age, per additional week | −0.08 (−0.18–0.01) | 0.077 |
| Birthweight percentile | 0.19 (−0.62–1.00) | 0.64 |
| BPD | 0.30 (0.02–0.58) | 0.035 |

Estimates were obtained using a multivariate linear regression.

large number of adult individuals born very preterm, in whom a very detailed birth history was collected and extensive clinical and biological parameters were recorded. This allowed us to identify, among our cohort, which individuals had a more severe neonatal course. Since we measured and did not observe any difference in PRA, Ang II, aldosterone or apelin, we were able to show that the elevation of copeptin is a selective hormonal consequence of preterm birth that has an even greater presence in case of a more severe neonatal course. Although this single timepoint study does not provide direct evidence that dysregulation of water balance is associated with CKD risk in individuals born preterm, it provides the basis for an original pathophysiological hypothesis that will be confirmed or denied by longitudinal studies.

## CONCLUSION

In conclusion, we found increased plasma copeptin levels, a surrogate marker for AVP levels, in young adults born preterm, especially in those with a more severe neonatal course. Increased copeptin levels are associated with smaller kidney volume and increased ACR, irrespective of RAAS activation and serum osmolality. As an increase in AVP levels may be involved in the occurrence and progression of a number of chronic diseases associated with preterm birth, these results pave the way for future longitudinal studies that will evaluate the prognostic value of plasma copeptin to predict the occurrence of disease, and possibly of interventional studies that will aim at decreasing AVP levels in this population.

## SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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## AUTHORS' CONTRIBUTIONS

T.M.L. and A.M.N. conceived the HAPI study and obtained the funding. A.F., T.M.L. and A.M.N. designed and performed the analysis and wrote the article. A.F., A.C., R.E.J. and R.A.S.S. collected the data. G.B. and A.L.L. participated in the interpretation of the results. All authors revised the manuscript.

## CONFLICT OF INTEREST STATEMENT

G.B. reports personal fees from Otsuka, outside the submitted work. All other authors have no conflicts of interest to disclose.

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