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Indoxyl Sulfate Contributes to Impaired Height Velocity in (Pre)School Children

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Introduction: Growth failure is considered the most important clinical outcome parameter in childhood chronic kidney disease (CKD). Central to the pathophysiology of growth failure is the presence of a chronic proinflammatory state, presumed to be partly driven by the accumulation of uremic toxins. In this study, we assessed the association between uremic toxin concentrations and height velocity in a longitudinal multicentric prospective pediatric CKD cohort of (pre)school-aged children and children during pubertal stages.

Methods: In a prospective, multicentric observational study, a selection of uremic toxin levels of children (aged 0–18 years) with CKD stage 1 to 5D was assessed every 3 months (maximum 2 years) along with clinical growth parameters. Linear mixed models with a random slope for age and a random intercept for child were fitted for height (in cm and SD scores [SDS]). A piecewise linear association between age and height was assumed.

Results: Data analysis included data from 560 visits of 81 children (median age 9.4 years; 2/3 male). In (pre) school aged children (aged 2–12 years), a 10% increase in concurrent indoxyl sulfate (IxS, total) concentration resulted in an estimated mean height velocity decrease of 0.002 SDS/yr ($P < 0.05$), given that CKD stage, growth hormone (GH), bicarbonate concentration, and dietary protein intake were held constant. No significant association with height velocity was found in children during pubertal stages (aged >12 years).

Conclusion: The present study demonstrated that, especially IxS contributes to a lower height velocity in (pre)school children, whereas we could not find a role for uremic toxins with height velocity during pubertal stages.

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Growth failure is considered the most important clinical outcome parameter in childhood CKD, because poor growth has been closely related to increased risk of hospitalization, poor school attendance, and death.[1–3] Moreover, growth failure has major long-term consequences on the child's quality of life, self-esteem, education level, level of employment, and chances of finding a partner.[4,5] Growth failure in childhood CKD remains common despite improvements in its general management. Depending on the country of residence and the proportion of children with kidney failure, a height below the third percentile has been reported in up to 45% of children.[6–9] Especially the growth outcomes of children on dialysis and post-transplantation did not significantly improve with time, whereas improved growth outcomes due to recent advantages appear to be limited to the predialysis period.[6,7] As a result, approximately 20% of patients with childhood-onset kidney failure have a final adult height likely to impact social integration and quality of life.[4–6]

Multiple factors have been identified to contribute to growth failure in childhood CKD, such as disturbances in the somatotropic hormone insulin-like growth factor-1 (IGF-1) axis, malnutrition, metabolic

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acidosis, CKD-mineral bone disease and vitamin D deficiency, delayed puberty, steroid therapy, and inflammation.[10-15] In particular, the chronic inflammatory state present in CKD is hypothesized to play a significant role in the pathophysiology of growth failure in childhood CKD. Inflammatory markers such as interleukin (IL)-1β and/or tumor necrosis factor-alpha via nuclear factor-kappa-B activation, have been related to the following: (i) an overexpression of suppressor of cytokine signaling proteins-2 (SOCS2) leading to an imbalanced Janus kinase/signal transducers and activators of transcription cascade and GH resistance, (ii) a decrease of chondrocyte proliferation, (iii) a decrease in appetite, and/or (iv) protein-energy wasting.[16-22] This chronic proinflammatory state is the result of oxidative stress; increased generation and reduced clearance of cytokines, infections, metabolic acidosis; accumulation of toxic organic metabolites (also called "uremic toxins") and advanced glycation end products; volume and sodium expansion, mineral bone disease; and concomitant diseases.[23,24]

In particular, the accumulation of uremic toxins due to decreased renal excretion whether or not accompanied by increased toxin generation, is recognized to contribute to this chronic proinflammatory state.[25,26] Up to now, more than 150 uremic retention solutes have been identified and they are divided into 3 categories, based on their physicochemical characteristics and their behavior during dialysis, as follows: (i) small, water-soluble compounds; (ii) middle molecules; and (iii) protein-bound uremic compounds. In particular the removal of protein-bound compounds is limited during dialysis because only the free unbound fraction of protein-bound uremic toxins can diffuse into the dialysate. When pathophysiological effects are demonstrated, the compounds are called uremic toxins. Uremic toxins have been related to many complications of kidney disease, especially cardiovascular and infectious diseases and the progression of kidney insufficiency, but also to a number of distressing patient-related outcomes, such as cognitive dysfunction or itching, which are not fatal but affect quality of life substantially.[25,27,28]

Despite extensive research on uremic toxicity over the past decades, the role of uremic toxins in CKD-related statural growth failure is unexplored. Nevertheless, several observational studies have demonstrated that a high weekly dose of hemodialysis induces catch-up growth in childhood kidney failure, suggesting that the enhanced uremic toxin removal in these extended hemodialysis strategies might contribute to improved growth.[29-32] Therefore, in this study, we explored the association between uremic toxin concentration and height velocity in a longitudinal cohort of children with different stages of CKD. We primarily aimed to assess the role of uremic toxins on the GH-dependent height velocity in (pre) school aged children (aged 2–12 years old), because inflammation is a crucial contributor in the pathophysiology of IGF-1 axis disturbances in CKD. Secondarily, we aimed to evaluate the role of uremic toxins on height velocity during pubertal growth (aged >12 years), in which height velocity is primarily dependent on the gonadotropic hormone axis.

METHODS

Study Population

In this cohort, children (aged 0–18 years) with CKD stages 1 to 5D were recruited from the Departments of Pediatric Nephrology of Ghent University Hospital, University Hospital Antwerp, University Hospital Leuven, CHC Liège, and University Hospital Saint-Luc Brussels between August 2014 and December 2017. CKD was defined according to the Kidney Disease: Improving Global Outcomes guidelines and classified in different stages (1–5D) according to estimated glomerular filtration rate, determined by the updated bedside Schwartz estimated glomerular filtration rate equation.[33] Exclusion criteria were active systemic inflammatory conditions (e.g., systemic lupus erythematosus) or active malignancy (e.g., posttransplant lymphoproliferative disease and malignancy under chemotherapy). Visits were performed away from active bacterial or viral infectious disease (e.g., urinary tract infections and respiratory infections) with implications for the child's wellbeing. Children with a genetic disease with an intrinsic impact on growth (i.e., Down syndrome) were excluded from this analysis. Study data were collected and managed using REDCap electronic data capture tools located on secure servers at the Ghent University Hospital.[34,35] REDCap is a secure, web-based software platform designed to support data capture for research studies, providing an intuitive interface for validated data capture, audit trails for tracking data manipulation and export procedures, automated export procedures for seamless data downloads to common statistical packages, and procedures for data integration and interoperability with external sources. The study protocol was approved by the Ethics Committee and written informed consent was obtained from all individual participants included in the study and/or from their parents (B670201524922; B670201422206).

Data Collection

Study visits were performed every 3 months for a maximum period of 2 years. Demographics were recorded at baseline, and clinical parameters (age,

anthropometric data, medical treatment, dialysis prescription, etc.) and dietary intake were recorded at each 3-monthly visit. Dietary protein intake was expressed as the achieved percentage of the recommended 100% dietary reference intake. For a detailed description of the dietary intake methodology, we refer to earlier published work by El Amouri *et al.*[36] 2021. Body composition monitoring (Fresenius Medical Care, St. Wendel, Germany) was performed at baseline, and subsequently once a year. All visits in this cohort that were performed in the first year after a kidney transplantation were excluded from this analysis. The following 2 growth outcome measures were selected: (i) height expressed in SDS according to World Health Organization Child Growth Standards (based on the chronological age of the child) and (ii) height expressed in cm.

Biochemical Measurements

From each participant, blood samples (ethylenediamine tetraacetic acid plasma and serum) were drawn every 3 months during a routine ambulatory visit. For the collection of serum, blood samples were allowed to clot for 20 to 30 minutes before centrifugation (2095*g*; 10 min; 4 °C). Serum and plasma aliquots were stored at −80 °C awaiting batch analysis. Standard laboratory assays at the clinical laboratory of the Ghent University Hospital (Ghent, Belgium) were used to measure serum biochemical parameters such as creatinine (Photometric [Architect c16000, Abbott, IL]), phosphate, calcium, parathyroid hormone, albumin and total protein. A selection of uremic toxins was made by the research team, based on the following: (i) previous published data,[25,37] (ii) the in-center expertise, and (iii) the physicochemical characteristics that affect the behavior of these toxins during dialysis. Concentrations of IxS, indole acetic acid, p-cresyl sulfate, p-cresyl glucuronide, hippuric acid and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid were quantified as previously described.[38] Briefly, for total concentrations, plasma samples were deproteinized by heat denaturation, followed by a filtration step through Amicon Ultra 0.5 ml filters (molecular weight cut-off 30 kDa, Millipore Merck, Darmstadt, Germany). For the free fraction, untreated plasma samples were filtered first through the Amicon Ultra Filters. Reversed-phase ultra-performance liquid chromatography (Agilent 1290 Infinity device, Agilent, Santa Clara, CA) was used to separate the uremic toxins. IxS (λ_{ex}: 280 nm, λ_{em}: 376 nm), *p*-cresyl sulfate and *p*-cresyl glucuronide (λ_{ex}: 264 nm, λ_{em}: 290 nm) and indole acetic acid (λ_{ex}: 280 nm, λ_{em}: 350 nm) were detected by an Agilent G1316C fluorescence detector. Hippuric acid and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid were detected with an Agilent G4212A diode array detector at 245 nm, and 254 nm, respectively. Plasma concentrations of the following solutes were determined by enzyme-linked immunosorbent assay (ELISA): symmetric dimethyl-arginine and asymmetric dimethyl-arginine (competitive ELISA from DLD Diagnostika, Hamburg, Germany), complement factor D (sandwich ELISA from R&D systems, Abingdon, UK) and β2M (sandwich ELISA from Orgentec Diagnostika, Germany). ELISAs were used according to the manufacturer's guidelines. ELISAs were analyzed using the EL808 Ultra Microplate Reader from Bio-Tek Instruments (Winooski, VT) using KC4V3.0 Analysis Software (Bio-Tek Instruments).

Uremic toxin concentrations were expressed as a concentration and as a z-score relative to the healthy control group (in SDS), using the reference values for healthy children published by Snauwaert *et al.*[39]. Three age categories (<6 years, 6–12 years, and 12–18 years) were used if uremic toxins were age-dependent (e.g., complement factor D, hippuric acid). The z-scores were calculated as follows: for child *i* the $z_i = (x_i - \overline{x_c}) / sd_{x_c}$; with x_i = concentration of uremic toxin in child i; $\overline{x_c}$ = average toxin level in the control group; and sd_{x_c} = SD of uremic toxin in the control group.[39] The z-scores can be interpreted in terms of SD away from the average toxin level in the healthy control group, independent of the effect of age.

Statistical Analyses

Continuous variables were summarized by their median value and (25th; 75th percentile). Categorical variables were described with absolute frequencies and percentages. Linear mixed models were fitted for height (SDS and cm). A piecewise linear model was selected to address the different stages of growth, that is, infants (aged <2 years) in which nutrition predominates growth, (pre)school age (2–12 years) in which IGF-1 axis disturbances predominates growth, and pubertal age (>12 years) in which sex hormones predominate growth. The fixed effects part included CKD stage (i.e., stage 1–2 vs. stage 3–4 vs. stage 5–5D, time-varying), GH (yes/no, time-varying), bicarbonate concentration (mmol/l; time-varying), within-patient mean dietary protein intake (% dietary reference intake, time-fixed), the respective concurrent toxin concentration (continuous, time-varying), age in years (piecewise linear with 2 knot locations at 2 and 12 years), and 2-way interactions between the toxin concentration and age splines. Models for height in cm were also adjusted for sex (time-fixed). The random effects part included a random slope for age and a random intercept for child. We assume a piecewise linear association between age and height, with 3

different (but connected) regression functions (Supplementary Figure S1A and B). If needed to improve linearity, the uremic toxin concentrations were naturally log-transformed. Statistical analyses were performed in R version 4.3.1.[40] Hypothesis testing was performed at the 2-sided 5% significance level.

RESULTS

Study Population

In Table 1, we summarize the patients' characteristics at baseline in the CKD1 to 5D cohort ($n = 81$ patients, 560 visits). For all children, between 2 and 10 visits were included. The median follow-up time of the patients in this observational cohort was 23.3 months; 75% of the patients in this cohort had a follow-up time of 15.6 months or more (Table 1). At baseline, 54% of children had an estimated glomerular filtration rate at baseline less than 30 ml/min per 1.73 m^2 and 15% were dialysis-dependent. Of the 560 visits, 123 were visits of patients treated with dialysis. The median age at baseline was 9.4 years (57% between 2 and 12 years, 36% >12 years) and 2 of 3 was male. Of all the visits, 309 were in the (pre)school age category (55%), whereas 231 (41%) during pubertal stages and only 20 (4%) during infancy. Half of the patients was diagnosed with congenital anomalies of kidney and urinary tract and 15% underwent a kidney transplant before the study. Dietary protein intake was adequate in this cohort, as reflected by the high average daily requirement index (i.e., the achieved percentage of the recommended 100% dietary reference intake) of ±180%, with a 25th percentile dietary reference intake of ≥100%. As visualized in Table 1, the children in this cohort had adequate nutritional state reflected by a normal serum albumin level. In addition, there was a controlled CKD-mineral bone disease (median parathyroid hormone was 65 ng/l) and metabolic acidosis (median bicarbonate level was 22.3 mmol/l). Recombinant GH (rGH) therapy use during ≥50% of study visits was present in 23% of the cohort. Children on rGH therapy were younger than children without rGH therapy (median age 6.6 years vs. 10.4 years) and had a more advanced stage of CKD (52% vs. 19% with estimated glomerular filtration rate less than 15 ml/min per 1.73 m^2 in the rGH vs. the non-rGH group) (Supplementary Table S1). Steroid use was limited to only low dose therapy (≤10 mg/m^2/d). In Supplementary Table S2, we summarize the baseline uremic toxin concentrations and z-scores.

Table 1. Patient characteristics at baseline in overall CKD stage 1-5D cohort

Characteristics	CKD stage 1–5D
Number of patients, *n*	81
Follow-up time (mo), median (25th pct; 75th pct)	23.3 (15.6; 25.2)
Total number of visits	560
Number of visits per patient, median (25th pct; 75th pct)	7 (5; 9)
Age (yr, at baseline), median (25th pct; 75th pct)	9.4 (4.1; 14.6)
Age category, *n* (%)	
<2 yr	6 (7%)
2–12 yr	46 (57%)
>12 yr	29 (36%)
Male, *n* (%)	52 (64%)
Renal diagnosis, *n* (%)	
CAKUT	41 (51%)
Cystic disease	3 (4%)
Glomerulonephritis	14 (17%)
Others/unknown	23 (28%)
CKD stage (at baseline), *n* (%)	
Stage 1–2 (eGFR >60 ml/min per 1.73 m^2)	16 (20%)
Stage 3 (eGFR 30–59 ml/min per 1.73 m^2)	21 (26%)
Stage 4 (eGFR 15–29 ml/min per 1.73 m^2)	22 (27%)
Stage 5 (eGFR <15 ml/min per 1.73 m^2)	10 (12%)
Stage 5D (PD + HD)	12 (15%)
Received dialysis (PD or HD) during or prior to the study?, *n* (%)	32 (40%)
Received a KTx prior to the study (all >1 yr before entry)?, *n* (%)	12 (15%)
Anthropometry (at baseline), median (25th pct; 75th pct)	
Height (SDS, at baseline)	−1.3 (−2.4; −0.4)
Calculated slope SDS/yr during study	0.1 (−0.2; 0.2)
Weight (SDS, at baseline)	−1.1 (−2.0; 0.0)
BMI (SDS, at baseline)[a]	−0.4 (−1.2; 0.4)
Body composition (at baseline), median (25th pct; 75th pct)[b]	
Lean tissue index (kg/m^2)	13.1 (12.3; 13.9)
Lean tissue mass (relative, %)	78.5 (70.0; 87.1)
Fat tissue index (kg/m^2)	3.4 (2.0; 5.9)
Fat tissue mass (relative, %)	14.9 (9.7; 22.7)
Dietary protein intake (relative to DRI %), median (25th pct; 75th pct)[c]	175 (118; 267)
Biochemistry (at baseline), median (25th pct; 75th pct)[c]	
Serum albumin (g/l)	44 (42; 47)
PTH (ng/l)	65 (41; 112)
25-OH vitamin D (ng/ml)	34 (23; 47)
Serum alkaline phosphatase (U/l)	220 (141; 283)
Serum calcium (mmol/l)	2.43 (2.35; 2.50)
Serum phosphate (mmol/l)	1.41 (1.27; 1.60)
Serum bicarbonate (mmol/l)	22.3 (20.2; 25.0)
Treatment (during study follow-up), *n* (%)	
rGH therapy (≥50% of study period)	19 (23%)
Steroid therapy (≥1 visit, all dose <10 mg/m^2/d)	7 (1%)
Cinacalcet	0 (0%)

BMI, body mass index; CAKUT, congenital anomalies of kidney and urinary tract; CKD, chronic kidney disease; DRI %, daily requirement index; eGFR, estimated glomerular filtration rate; HD, hemodialysis; i.e., achieved percentage of the recommended 100% dietary reference intake; KTx, Kidney transplantation; mo, months; pct, percentile; PD, peritoneal dialysis; PTH, parathyroid hormone; rGH, recombinant growth hormone therapy; SDS: SD score.
[a] $n = 76$ (5 missing <2 yr of age at baseline)
[b] $n = 71$ (10 missing, children <15 kg)
[c] $n = 72$ (9 missing)

Primary Outcome: Height Velocity in (Pre) School Aged Children (2–12 years)

In Table 2, we summarize the results related to the outcome height velocity (expressed in SDS/yr). For every 10% increase in concurrent creatinine, IxS (total and free), *p*-cresyl sulfate (total and free) concentration, the estimated mean height velocity decreases with

Table 2. The impact of the interaction between uremic toxins and age on height in (pre)school aged children between 2 and 12 years (in SDS and in cm)

Age category: 2–12 yr	Heigh velocity (SDS/yr)	Height velocity (cm/yr)
Visits	309	309
Small water soluble molecules		
SDMA (μg/l)[a]	−0.00096 (−0.0035; 0.0016)	−0.00075 (−0.011; 0.0094)
ADMA (μg/l/10,000)	1.5 (−1.1; 4)	2.7 (−8.5; 14)
Phosphate (mmol/l/10)	−0.017 (−0.34; 0.29)	0.75 (−0.59; 2.1)
Creatinine (mg/dl)[a]	**−0.0039 (−0.0074; −0.00033)**[b]	−0.0097 (−0.027; 0.0078)
Middle molecules		
β2-microglobulin (μg/ml)[a]	0.00027 (−0.0022; 0.0029)	−0.0023 (−0.013; 0.0086)
Complement factor D (mg/dl)[a]	0.00075 (−0.0042; 0.0058)	**0.031 (0.0052; 0.056)**[b]
Parathyroid hormone (ng/l)[a]	−0.0011 (−0.0024; 0.000088)	−0.0037 (−0.0087; 0.0012)
Protein-bound uremic toxins		
p-cresyl glucuronide (total, mg/dl)[a]	−0.0007 (−0.0015; 0.00014)	−0.0021 (−0.0056; 0.0014)
p-cresyl glucuronide (free, mg/dl)[a]	−0.0004 (−0.0012; 0.00041)	−0.0013 (−0.0046; 0.002)
Hippuric acid (total, mg/dl)[a]	−0.0000 (−0.0009; 0.0001)	0.00043 (−0.0036; 0.0044)
Hippuric acid (free, mg/dl)[a]	0.00046 (−0.00057; 0.0015)	0.001 (−0.0033; 0.0054)
Indole acetic acid (total, mg/dl)[a]	0.0012 (−0.00061; 0.0029)	0.0032 (−0.0045; 0.011)
Indole acetic acid (free, mg/dl)[a]	0.00067 (−0.00033; 0.0017)	0.0015 (−0.0027; 0.0058)
Indoxyl sulfate (total, mg/dl)[a]	**−0.0023 (−0.0036; −0.00086)**[b]	**−0.0063 (−0.012; −0.00054)**[b]
Indoxyl sulfate (free, mg/dl)[a]	**−0.0015 (−0.0025; −0.00041)**[b]	−0.0046 (−0.009; −0.00013)
p-cresyl sulfate (total, mg/dl)[a]	**−0.0012 (−0.0022; −0.0002)**[b]	−0.0036 (−0.0078; 0.00062)
p-cresyl sulfate (free, mg/dl)[a]	**−0.0014 (−0.0025; −0.00034)**[b]	−0.004 (−0.0085; 0.00043)
CMPF(mg/dl)[a]	0.00073 (−0.00021; 0.0017)	−0.083 (−0.17; 0.003)

ADMA, asymmetric dimethyl-arginine; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; SDMA, symmetric dimethyl-arginine; SDS, SD score.
[a]Natural log-transformed to improve linearity, results correspond to a 10% increase in toxin concentration.
[b]Boldface: *P*-value < 0.05.
Results of linear mixed models fitted for height (in SDS and in cm) in (pre) school aged children (2–12 yr).

0.001 to 0.004 SDS/yr ($P < 0.05$), given that CKD stage, GH, bicarbonate concentration, and dietary protein intake are held constant. No significant association with height velocity was found for the other assessed uremic toxins (Table 2).

Given that the use of SDS for height is based on the chronological age of the child and does not allow for the expectation that growth trajectories may be inherently different in CKD, the analysis was repeated with the outcome height velocity expressed in cm/yr to confirm our findings. As visualized in Table 2, similar results for IxS (total) were obtained. In contrast, no significant association with height velocity was found for *p*-cresyl sulfate and creatinine.

Secondary Outcomes: Height Velocity in Pubertal Stages (Children Aged >12 Years)

In contrast to the (pre)school age category, no association between height velocity (SDS/yr) and uremic toxin concentrations were found (Table 3). When the outcome height velocity was expressed in cm/yr, an inverse association with complement factor D and a positive association with parathyroid hormone was found (Table 3).

DISCUSSION

This study explored the association of uremic toxin concentrations and height velocity in (pre)school aged children (2–12 years) and adolescents (>12 years) with a variable degree of kidney impairment, using a unique longitudinal cohort to address the well-described intra-patient variability of uremic toxin concentrations.[41] In the present study, we found the following: (i) that higher concentrations of especially IxS are associated with a decreased height velocity in (pre)school children, and (ii) that the height velocity in pubertal stages was not associated with uremic toxin concentrations.

First, our study found that in (pre)school children, higher concentrations of especially IxS were associated with a decrease in the estimated mean height velocity, irrespective of CKD stage, GH, metabolic acidosis correction, and dietary protein intake. Linear growth in (pre)school aged children is predominantly dependent on the somatotrophic GH/IGF-1 axis.[15] Disturbances in the GH/IGF-1 axis signaling pathways are commonly found in children with chronic inflammatory conditions such as juvenile idiopathic arthritis, celiac disease, and CKD.[42,43] In CKD, a role for IxS is hypothesized from experimental studies in the pathophysiology of GH/IGF-1 axis signaling pathway disturbances as a result of an imbalanced JAK2-STAT5 signaling pathway.[44] Although the cellular mechanisms by which JAK2-STAT5 signaling pathway is impaired in CKD remains incompletely characterized, SOCS2 overexpression is identified to play a crucial role in its pathophysiology.[14] SOCS2 is a critical negative

Table 3. The impact of the interaction between uremic toxins and age on height in adolescents during pubertal stages (>12 years) (in SDS and in cm)

Age category: >12 yr	Heigh velocity (SDS/yr)	Height velocity (cm/yr)
Visits	231	231
Small water soluble molecules		
SDMA (μg/l)[a]	−0.00011 (−0.0073; 0.007)	−0.011 (−0.039; 0.018)
ADMA (μg/l/10,000)	−1.3 (−6.3; 3.5)	0.72 (−20; 21)
Phosphate (mmol/l/10)	−0.13 (−0.64; 0.38)	**−0.63 (−2.8; 1.6)**[b]
Creatinine (mg/dl)[a]	0.00094 (−0.0041; 0.006)	**0.0016 (−0.024; 0.028)**[b]
Middle molecules		
β2-microglobulin (μg/ml)[a]	−0.0037 (−0.0082; 0.00078)	−0.0099 (−0.032; 0.012)
Complement factor D (mg/dl)[a]	−0.0048 (−0.013; 0.0034)	−0.063 (−0.11; −0.015)[b]
Parathyroid hormone (ng/l)[a]	0.0015 (−0.00059; 0.0036)	0.01 (0.0016; 0.019)[b]
Protein-bound uremic toxins		
p-cresyl glucuronide (total, mg/dl)[a]	0.000038 (−0.0014; 0.0015)	0.0017 (−0.005; 0.0086)
p-cresyl glucuronide (free, mg/dl)[a]	−0.00026 (−0.0016; 0.0011)	0.0015 (−0.0047; 0.0077)
Hippuric acid (total, mg/dl)[a]	−0.00058 (−0.0022; 0.001)	−0.0023 (−0.0097; 0.0051)
Hippuric acid (free, mg/dl)[a]	−0.0012 (−0.0028; 0.00033)	−0.0032 (−0.01; 0.0036)
Indole acetic acid (total, mg/dl)[a]	0.00045 (−0.0022; 0.0031)	0.00044 (−0.011; 0.012)
Indole acetic acid (free, mg/dl)[a]	−0.0004 (−0.0019; 0.0011)	−0.0025 (−0.0092; 0.0043)
Indoxyl sulfate (total, mg/dl)[a]	0.00014 (−0.0022; 0.0025)	0.0001 (−0.01; 0.011)
Indoxyl sulfate (free, mg/dl)[a]	−0.00014 (−0.0018; 0.0015)	0.00014 (−0.0072; 0.0074)
p-cresyl sulfate (total, mg/dl)[a]	0.00045 (−0.001; 0.0019)	0.0023 (−0.0039; 0.0084)
p-cresyl sulfate (free, mg/dl)[a]	0.00051 (−0.0013; 0.0023)	0.0047 (−0.003; 0.012)
CMPF (mg/dl)[a]	−0.00051 (−0.002; 0.00094)	0.00047 (−0.0059; 0.0067)

ADMA, asymmetric dimethyl-arginine; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; SDMA, symmetric dimethyl-arginine; SDS, SD score.
[a]Natural log-transformed to improve linearity, results correspond to a 10% increase in toxin concentration.
[b]Boldface: *P*-value < 0.05.
Results of linear mixed models fitted for height (in SDS and in cm) in adolescents during pubertal stages (>12 yr).

regulator of GH-stimulated JAK2-STAT5 signaling pathway and can, independently of IGF-1, inhibit the growing bone.[16,20,44-46] Known stimulators of SOCS2 relevant in the context of CKD are proinflammatory cytokines (i.e., IL-6, IL-1, tumor necrosis factor-alpha) but also IxS has been directly linked to SOCS2 overexpression by binding the aryl hydrocarbon receptor.[47,48] In addition, IxS is an important contributor to the chronic proinflammatory state present in CKD, by inducing the release of IL-1β and several other proinflammatory cytokines.[26,49]

To the best of our knowledge, this is the first clinical study that found an association between IxS and height velocity in (pre)school aged children**.** The role of IxS and other protein-bound uremic toxins has been hypothesized by several clinical studies addressing extended hemodialysis strategies and residual kidney function. Previous research has taught us that the high degree of protein-binding of IxS (and other protein-bound uremic toxins) strongly limits the removal of these toxins during both diffusive and convective dialysis strategies, and that the removal of strongly bound uremic toxins such as IxS is predominantly related to the amount of processed blood, independent of the time frame used to obtain these volumes.[50] Observational studies have demonstrated that a high weekly dose of hemodialysis induces catch-up growth in childhood kidney failure, suggesting that the enhanced uremic toxin removal in these extended hemodialysis strategies might contribute to improved growth.[29-32] However, major changes in protein-bound uremic toxin concentrations after transitioning from conventional HD to an extended HD prescription could not be found by several studies in adult population, whereas Meyer *et al.*[51] 2016 could only detect a −11 (−6%; −15%) reduction in IxS in the HEMO study, no reduction was found in the concentrations of protein-bound uremic toxins in the Frequent Hemodialysis Network Daily Trial[52] and the observational study by Kalim *et al.*[53] 2018. We could not find an association between height velocity and middle molecules in both (pre)school children and adolescents. This is in contrast to the hemodiafiltration Heart Height study that found a static annualized change in height SDS in the purely diffusive conventional HD group whereas a significant (modest) increase in the annualized change in height SDS was found in children treated with posthemodiafiltration, a combined diffusive and convective therapy that is proven effective in decreasing predialysis levels of middle molecules such as parathyroid hormone and β2m.[54-60] Additional studies in pediatric populations are needed to further address the mechanisms behind the beneficial effects of extended hemodialysis strategies on growth.

In addition, studies assessing the relationship between growth failure and residual kidney function have suggested a role for protein-bound uremic toxins in its pathophysiology, because the concentrations of IxS

(and other protein-bound uremic toxins) are closely related to the degree of residual kidney function on maintenance dialysis.[50,56,61-63] Residual kidney function has previously been associated with height velocity in children on peritoneal dialysis, whereas the authors could not find an association between height velocity and small-molecule clearance during peritoneal dialysis.[64]

Second, our study found no association between height velocity and uremic toxins during pubertal stages. The pubertal growth spurt is typically delayed in adolescents on dialysis due to an insufficient activation of the hypothalamic GnRH pulse generator, resulting in loss of growth potential.[65-67] Experimental studies have taught us that inflammatory cytokines can have a direct effect on the hypothalamic-pituitary gonadal axis, that is, both lipopolysaccharide and IL-1 administration suppressed the secretion of both luteinizing hormone-releasing hormone and luteinizing hormone in female rats.[68-71] To the best of our knowledge, this is the first clinical study that assessed the role of uremic toxins and height velocity during pubertal stages. More experimental and clinical studies are needed to further explore and understand the pathophysiology of pubertal development disturbances during CKD and confirm the findings in the present study.

Although this study is the first to assess the association of uremic toxins and height velocity in children with CKD, our study has some limitations. First, the heterogenicity of the rather small cohort of children might have hampered us to find an association between uremic toxin concentrations and height velocity. Indeed, the cohort included children with diverse types of kidney disease and a wide range of kidney impairment. Nevertheless, the fact that this multicentric study was performed in solely 1 country with an established national multidisciplinary program for children with CKD including standard reimbursed dietary follow-up and uniform access to rGH therapy and tube feeding, allowed us to minimize the recognized impact of regional differences in the management of growth in childhood CKD.[72] Second, whereas the study design prioritized the incorporation of the longitudinal accumulation of uremic toxins with a large intrapatient variability, only a selection of uremic toxins was assessed in this study, and the rather short median observational period of 2 years might have enabled us to find an association with height velocity. Lastly, data on Tanner stages to exactly define the cut-off between the (pre)school aged children and the children during pubertal stages was not available and was subsequently arbitrarily fixed at 12 years of age, which might have hampered us to find associations between uremic toxin concentrations and height velocity during pubertal stages.

In conclusion, we hypothesize that, especially IxS contributes to a lower height velocity in (pre) school children, whereas we could not find a role for uremic toxins on height velocity during pubertal stages. The hypotheses formulated from this observational study need to be translated into future experimental studies to identify which and how uremic toxins are contributing to the pathophysiology of GH/IGF-1 and hypothalamic-pituitary gonadal signaling pathway disturbances. Moreover, efforts are needed to identify the uremic-toxin driven molecular signaling pathways causing GH/IGF-1 resistance and hypothalamic-pituitary gonadal axis disturbances in the search for more targeted strategies. These targeted therapies may offer a more physiological correction of height velocity and ideally prevent growth impairment in future.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. (A) Piecewise linear association between age and height (in SDS), with 3 different (but connected) regression functions. (B) Piecewise linear association between age and height (in cm), with 3 different (but connected) regression functions.

Table S1. Patient characteristics of patients with growth hormone therapy versus no growth hormone therapy in the overall CKD1-5D cohort.

Table S2. Concentrations of selected uremic toxins at baseline and during the study in the overall CKD stage 1-5D cohort.

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