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Growth trajectories and kidney function in early childhood

Maria Margarida Carvalhais Alves Dias

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RESUMO

Introdução e objetivos: O crescimento pós-natal desempenha um papel importante no desenvolvimento da criança, possivelmente tendo impacto no risco de obesidade tardia e doenças cardiovasculares e renais. O objetivo do presente trabalho foi estudar a associação entre diferentes trajetórias de crescimento e a função renal de crianças pré-púberes.

Métodos: Avaliação transversal de 1004 crianças com 8-9 anos, divididas em 4 grupos com diferentes trajetórias de crescimento, do nascimento aos 6 anos de idade. A taxa de filtração glomerular (TFG) foi estimada usando valores séricos de creatinina e/ou cistatina C, através das fórmulas de Schwartz revista, Filler, LeBricon, CKD-EPI combinada, Zappitelli combinada, Schwartz combinada e CKiD combinada em toda a amostra, e medida pela clearance de creatinina (ClCr) de 24-horas, numa subamostra de 261 crianças. Os resultados foram comparados entre crianças com ganho ponderal normal (trajetória I, n=640), persistente (trajetória II, n=117), durante a infância (trajetória III, n=161) e durante os primeiros 2.5 anos de vida (trajetória IV, n=86).

Resultados: A trajetória I incluiu os indivíduos com os menores valores de creatinina sérica e cistatina C e os valores mais elevados de TFG, enquanto a trajetória II incluiu os indivíduos com os menores valores de TFG (média±desvio padrão, DP), em mL/min/1.73m^2, segundo as diversas fórmulas: Filler 150±18 vs. 145±15 (p=0.002), Le Bricon 125±13 vs. 122±11 (p=0.002) e CKD-EPI 154±10 vs. 151±9 (p=0.005). A trajetória I incluiu os indivíduos com os menores valores de ClCr, enquanto a trajetória II incluiu os indivíduos com os valores mais elevados: 98±24 vs. 115±23 mL/min (p=0.005). Nos modelos de regressão linear multivariada (ajustados para idade, sexo, classe de peso ao nascimento, pressão arterial sistólica e índice de massa corporal), a trajetória III foi associada a valores 2.68 a 3.75 mL/min/1.73m^2 menores de TFG, nos dois modelos que incluíram as fórmulas baseadas apenas na cistatina C, Le Bricon e Filler, respetivamente, do que na trajetória I. As crianças na trajetória III também apresentaram TFG 2.06 (intervalo de confiança 95%: -3.74 a -0.39) mL/min/1.73m^2 (p=0.016) menor, estimada pela fórmula CKD-EPI, quando comparadas com as crianças na trajetória I.

Conclusões: O nosso trabalho corrobora a influência dos padrões de crescimento na função renal tardia ao longo da infância, o que provavelmente se deve à ação de múltiplos mecanismos deletérios associados ao excesso de peso desde idades precoces. As diferenças encontradas na função renal, apesar de subtis, podem traduzir-se em

importantes diferenças na sobrevida renal a longo prazo e na incidência de doença renal crónica ao longo da vida, tornando evidente a necessidade de desenvolver estratégias de prevenção da obesidade desde o início da vida.

Palavras-chave: Infância; Crianças; Crescimento; Trajetórias de Crescimento; Função Renal; Obesidade Infantil; Ganho Ponderal.

ABSTRACT

Background and objectives: Postnatal growth plays a major role in the future development of children, possibly having an important impact on the risk of later obesity, cardiovascular and kidney disease. In the present study, we aimed to study the association between different trajectories of weight gain during infancy and early childhood and the renal function of prepubertal children.

Methods: Cross-sectional study of 1004 children aged 8-9 years, divided in 4 groups with different trajectories of weight gain from birth to 6 years. Glomerular filtration rate (GFR) was estimated from serum creatinine and cystatin C using Revised Schwartz, Filler, LeBricon, Combined CKD-EPI, Combined Zappitelli, Combined Schwartz and Combined CKiD formulas, in all sample, and measured by 24-hour creatinine clearance (CrCl), in a subsample of 261 children. The results were compared between children with normal weight gain (trajectory I, n=640), persistent weight gain (trajectory II, n=117), weight gain during childhood (trajectory III, n=161) and weight gain during infancy (trajectory IV, n=86).

Results: Trajectory I included the individuals with the lowest values of serum creatinine and cystatin C and the highest values of eGFR (estimated GFR), while trajectory II included the individuals with the lowest values of eGFR (mean±standard deviation, SD) values, in mL/min/1.73m^2: by Filler 150±18 vs. 145±15 (p=0.002), by Le Bricon 125±13 vs. 122±11 (p=0.002) and by CKD-EPI 154±10 vs. 151±9 (p=0.005). Trajectory I included the individuals with the lowest values of absolute CrCl while trajectory II included those with the highest values: 98±24 vs. 115±23 mL/min (p=0.005). In multivariate linear regression models (adjusted for age, sex, class of birthweight for gestational age, systolic blood pressure and current body mass index), trajectory III was associated with a 2.68 to 3.75 lower eGFR, in the 2 models including the cystatin C-based formulas of Le Bricon and Filler, than in trajectory I. In addition, children in trajectory III also presented a 2.06 (95% confidence interval: -3.74 to -0.39) mL/min/1.73m^2 (p=0.016) lower eGFR, estimated by CKD-EPI, when compared to those in trajectory I.

Conclusions: Our work supports the influence of childhood growth patterns in later kidney function, which is probably due to the action of multiple deleterious mechanisms associated with excess weight accumulation from early ages. The differences found on kidney function, despite being subtle, might translate in important differences on the long-term renal survival and on the life course cumulative incidence of chronic kidney disease, thus calling for a reinforcement of preventive strategies to obesity prevention from early in life.

Key words: Childhood; Children; Growth; Growth Trajectory; Kidney Function; Pediatric Obesity; Weight Gain.

ABBREVIATIONS

AGA, adequate for gestational age

BMI, body mass index

BP, blood pressure

BSA-IBW, body surface area calculated by Haycock equation using ideal body weight instead of child's real weight

BSA, body surface area

CI, confidence intervals

Cr, creatinine

CrCl, 24-hour creatinine clearance

CysC, cystatin C

DBP, diastolic blood pressure

eGFR, estimated glomerular filtration rate

GFR, glomerular filtration rate

IBW, ideal body weight

LGA, large for gestational age

SBP, systolic blood pressure

SD, standard deviation

SGA, small for gestational age

WHO, World Health Organization

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INTRODUCTION

Postnatal growth is influenced by many factors and plays a major role in the future development of children. There are 3 phases of postnatal growth: infancy (from birth to 2 years old), childhood (from 2 to puberty) and pubertal period [1]. Monitoring growth and development of a child allows us to identify the groups at greatest risk and in need for specific interventions, aiming to reduce infant morbidity, as well as to intervene in outcomes that these children may present later in life, improving chronic disease prevention [2].

Childhood obesity has become a global epidemic in the last decades with well-known associations with several adverse health outcomes in later childhood and adulthood. Being overweight or obese is now considered a public health problem [3]. Moreover, many studies have established that childhood growth patterns might have an important impact on the risk of later obesity and cardiovascular disease throughout life [4]. Rapid weight gain in the first 2 years of life, as well as excessive weight before puberty, have been shown to be associated with earlier pubertal maturation both in boys and girls and with a higher risk of obesity, diabetes, hypertension and cardiovascular disease later in life [5,6]. Previous studies have found that growth trajectories with early excessive weight gain are associated with thicker and stiffer arteries in childhood, reflecting a worst vascular profile [7]. Another study reported that accelerated weight gain during childhood and puberty was associated with a higher risk of coronary events in later adult life [8].

In the recent years, evidence has suggested that the rise in the prevalence of obesity has paralleled the rise in the incidence of kidney disease [9], suggesting that obesity is an independent risk factor for chronic kidney disease not only in adults [10] but also in children [11]. In a previous study of our group, we reported that overweight and obese children aged 8 and 9 years old already have altered renal function when compared to their non-overweight counterparts [12]. However, few studies have examined the impact of rapid weight gain, both in infancy and in childhood, in later kidney function.

In the present study, we aim to study the association between different trajectories of weight gain during infancy and early childhood and the renal function of prepubertal children. We will compare several glomerular filtration rate (GFR) estimations based on creatinine (Cr) and cystatin C (CysC) values, in children aged 8-9 years, distributed in 4 groups of weight gain trajectories from birth to 6 years.

METHODS

Study design and sample

We studied children aged 8-9 years that have been followed since birth in a previously established birth cohort study in Porto, Portugal (Generation XXI) [13]. From the original cohort (n=8647), 4590 children attended a face-to-face follow-up visit at 7 years of age, including anthropometric evaluation and blood sample collection, thus being eligible for the ObiKid project – a specific project aiming to clarify the impact of childhood obesity and associated co-morbidities on the kidney [12]. A random group of 1093 children (computer-generated random numbers simple randomization) was preselected to be screened for serum Cr and CysC, but for the present study we excluded 89 children: 50 children excluded due to missing data on Cr and 39 children due to impossibility to estimate a growth trajectory (insufficient number of anthropometric measurements abstracted from previous records). Therefore, we finally included 1004 participants in the present analysis. We were able to assess 24-hour creatinine clearance (CrCl) in 261 randomly selected children, from the 1004 children included in the present analysis.

Data collection and variable definition

The study visits took place at the Public Health and Forensic Sciences and Medical Education Department, Faculty of Medicine, University of Porto. Data on children and family medical history was collected by the application of a questionnaire and perinatal information, such as gestational age and anthropometry at birth, had already been abstracted from medical records in earlier evaluations. Children's classes of gender-specific birthweight for gestational age were defined according to the revised Fenton growth charts [14].

Anthropometric and general physical examinations were performed, including weight, height and waist circumference and body composition assessed by foot-to-foot bioelectrical impedance analysis (Tanita®, model TBF-300). Body mass index (BMI) was calculated (kg/m^2) and BMI-for-age values were classified according to the World Health Organization (WHO) reference data for BMI z-score into the following categories: normal weight (≤ +1 standard deviations, SD), overweight (> +1 SD and ≤ +2SD) and obesity (> +2 SD) [15].

Blood pressure (BP) was measured in all children at the right arm using an aneroid sphygmomanometer (Elite 92125, Medel®, Italy), with a cuff size appropriate to the child's arm circumference. BP measurements were taken three times with a 1-minute interval, by a trained examiner, with the child in a seated position and the antecubital fossa supported at heart level, after at least a 5-minute rest. The second and third measurements were averaged for analysis.

Laboratory procedures

Venous blood samples were collected after an overnight fast of at least 8 hours and analyzed for Cr and CysC. All the laboratory analyzes were performed in the Clinical Pathology Department of Centro Hospitalar Universitário São João, Porto – Portugal. The serum Cr assay was based on the Jaffé compensated traceable to an isotope dilution mass spectrometry method (Olympus AU 5400 automated analyzer, Beckman-Coulter®, USA) [16]. Urinary Cr was determined with the same clinical chemistry analyzer. Serum CysC was assayed using a particle-enhanced immunonephelometric assay (N latex Cystatin C, Siemens®, Germany). To estimate GFR, in mL/min/1.73 m^2, the following formulas were used: Revised Schwartz formula, GFR = k x (height/serum Cr); k = 0.413 [17]; Filler formula, Log(GFR) = 1.962 + (1.123 x log(1/CysC)) [18]; LeBricon formula, GFR = (78/CysC) + 4 [19]; Combined CKD-EPI formula, GFR = 135 x min(serum Cr/k, 1)$^{\alpha}$ x max(serum Cr/k, 1)$^{-0.601}$ x min(CysC/0.8, 1)$^{-0.375}$ x max(CysC/0.8, 1)$^{-0.711}$ x 0.995^{age} x 0.969 (if female) x 1.08 (if black), k = 0.7 (females) or 0.9 (males), α = -0.248 (females) or -0.207 (males) [20]; Combined Zappitelli formula, GFR = (507.76 x $e^{0.003 \times height}$)/(CysC$^{0.635}$ x serum Cr$^{0.547}$) [21]; Combined Schwartz formula, GFR = 39.8 x (height/serum Cr)$^{0.456}$ x (1.8/CysC$^{0.418}$ × (30/blood urea nitrogen)$^{0.079}$ x (height/1.4)$^{0.179}$ (x1.076 if females) [22]; Combined CKiD formula, GFR = 39.8 x (height/serum Cr)$^{0.456}$ x (1.8/CysC)$^{0.418}$ x (30/blood urea nitrogen)$^{0.079}$ x (1.076 if male) or (1.00 if female) x (height/1.4)$^{0.179}$ [23]. Serum Cr is expressed in mg/dL; CysC is expressed in mg/L; height is expressed in cm; age is expressed in years; blood urea nitrogen is expressed in mg/dL.

The 24-hour CrCl (in mL/min) was calculated according to the standard formula and was considered to be the absolute CrCl. The absolute CrCl was then multiplied by 1.73 and divided by the child's body surface area (BSA) (determined by the Haycock formula [24]), in order to obtain the standard value of CrCl in mL/min/1.73 m^2 (normalized to 1.73 m^2 of body surface). BSA using ideal body weight (IBW) (in kg), instead of child's actual weight, was used as an alternative GFR adjuster. The use of IBW-derived BSA for GFR normalization has been postulated previously [25] and recently also found to avoid overcorrection and GFR underestimation, both in adult and pediatric studies [26,27]. Moreover, it has been found to be equally closely correlated to the absolute GFR in overweight/obese children, with the advantage of allowing comparisons with the standard GFR values [28]. The IBW was inferred by calculating weight based on the 50th percentile of BMI-for-age: IBW = BMI at 50th percentile (kg/m^2) x squared height (m) [29].

Oral and written information on the correct methods of 24-hour urine collection were given to all children's caretakers and compliance was rechecked by a quick questionnaire as the samples were delivered. In order to consider the 24-hour urine samples valid, urinary Cr

had to be between 11.3 and 28.0 mg/kg/day (according to age- and sex-specific reference values [30]) and urinary volume had to be over 300 mL.

Growth trajectories definition

The definition of growth trajectories for the participants of Generation XXI was based on an extensive body of data of anthropometric measurements abstracted from the children's health books, recorded in routine care, from birth until the age of 6 years. For the final analysis and growth trajectories definition, some weight records were considered implausible and excluded (>4 or <-4 SD from the mean) and all individuals with less than 5 weight measurements were also excluded. Furthermore, when more than 2 weight measurements were recorded in a month, the mean of those measurements was considered to attenuate autocorrelation. In the final estimation of growth trajectories, a total of 86428 weight measurements (80.8% of all values abstracted) from 5237 children were considered, with a median of 16 (25^{th}-75^{th} percentile (P25-P75): 13-19) weight measurements records available per child. The growth trajectories patterns were defined by the intercept, slope, quadratic and cubic random terms estimated by a mixed model (Normal Mixture Modeling for Model-Based Clustering). The most appropriate models were those that allowed the best homogeneous grouping of the individual patterns of growth. This method for growth curve modeling was described previously [31,32].

Finally, four different growth trajectories for both sexes together were defined, that included children with similar growth patterns over time. For illustrative purposes and to relate the Generation XXI trajectories with universally used growth charts, the growth trajectories were plotted over the WHO growth charts (Figure 1). The four trajectories were labeled as "normal weight gain" (trajectory I), "persistent weight gain" (trajectory II), "weight gain during childhood" (trajectory III) and "weight gain during infancy" (trajectory IV). For simplicity, we will further refer to periods of weight change as infancy and childhood, the former including children aged less than 30 months (the first 2.5 years of life). Children in trajectory II diverged immediately after birth, showing a rapid increase in weight gain during the first 10 months of life. After this period, this trajectory exhibited a consistent weight gain pattern, the highest during the entire period of analysis, thus including the heaviest children of our sample. Children in trajectory I exhibited a consistent percent of weight gain, the slowest during the period analyzed, including the lighter children. Trajectories I and III showed a similar weight gain pattern until 20 months of age but then trajectory III diverged and children presented a higher increase of weight gain until the end of the analyzed period (the second heaviest group of children after 30 months). Finally, trajectory IV included the smallest babies at birth but with higher weight gain levels until 10 months of age, being the

second trajectory with heaviest children in this early infancy period. For purposes of analysis, all the trajectories will be compared to trajectory I, considered the closest to the standard and desirable pattern of growth in childhood.

The 1004 children in the current study sample were distributed by growth trajectories similarly to the remaining sample: 63.7%, 11.7%, 16.0% and 8.6% versus 62.1%, 11.7%, 16.2% and 10.0% in trajectories I, II, III and IV, respectively, p=0.551. At birth, they were slightly longer (49.0 versus 48.7 cm, p<0.01) and heavier (3236 versus 3181 g, p=0.01) possibly due to a slightly higher gestational age (38.8 versus 38.6 weeks, p<0.01). Also, they presented lower values of systolic blood pressure (SBP) (97.5 vs 98.2 mmHg, p=0.019) and diastolic blood pressure (DBP) (57.0 vs 57.9 mmHg, p=0.08) at 4 years of age, but there were no differences in height, weight and BMI.

Ethics

The ObiKid project was approved by the Ethics Committee of Centro Hospitalar Universitário São João, E.P.E., Porto – Portugal and Faculty of Medicine of the University of Porto. It complies with the Helsinki Declaration, the guidelines for the ethical conduct of medical research involving children [33] and the current national legislation. Written informed consent from parents or their legal substitute and verbal assent from children was obtained, concerning information and biological samples gathering.

Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics 25.0. The one-way analysis of variance (ANOVA) test and chi-square tests were performed in order to analyze the differences in continuous and categorical variables between trajectories, respectively. Linear multivariate regression models were used to determine the effect of each trajectory on renal function markers and eGFR (estimated glomerular filtration rate); trajectory I was used as reference. These models were adjusted for sex and children's current age, class of birthweight for gestational age, SBP z-score and class of BMI (3 categories – normal weight, overweight and obese). Data are presented as linear regression coefficients (β) and 95% confidence intervals (CI). All p values are two-sided and were considered statistically significant if <0.05.

RESULTS

A total of 1004 children (50.8% male) with a mean (SD) age of 7.5 (0.8) years were included in the present study.

General characteristics of the study sample by growth trajectories are presented in Table I. At this age, 22.3% of the children were categorized as overweight and 15.9% as obese. Among overweight and obese children, the mean (SD) BMI z-score was 1.53 (0.29) and 2.68 (0.49), respectively. There were several differences in both current and birth anthropometrics among trajectories depicted in Table I. Trajectory I included children with the lowest mean values of weight z-score, BMI z-score and SBP z-score and the highest gestational age; the highest percentage of normal weight children was found in this trajectory. On the other hand, trajectory II was associated with the highest mean values of weight z-score, BMI z-score, SBP z-score, DBP z-score and birthweight. The second highest mean values of weight z-score and BMI z-score were found in trajectory III. Trajectory IV presented the lowest mean values of DBP z-score.

Table II presents renal function markers and eGFR values by growth trajectories. There were differences among the different trajectories in the mean levels of serum Cr and CysC, GFR (estimated by Filler, Larsson, Le Bricon and CKD-EPI formulas) and in the mean levels of absolute CrCl; trajectory I included the individuals with the lowest values of serum Cr and CysC and the highest values of eGFR, while trajectory II included the individuals with the lowest values of eGFR: by Filler 150±18 vs. 145±15 mL/min/1.73m^2 (p=0.002), by Le Bricon 125±13 vs. 122±11 mL/min/1.73m^2 (p=0.002) and by CKD-EPI 154±10 vs. 151±9 mL/min/1.73m^2 (p=0.005). Trajectory I included the individuals with the lowest values of absolute CrCl while trajectory II included the individuals with the highest values: 98±24 vs 115±23 mL/min (p=0.005). In addition, children in trajectory I presented the highest values of BSA-adjusted 24h-CrCl whereas those in trajectory II presented the lowest values but the difference was not statistically significant. The opposite was verified when CrCl was adjusted to BSA-IBW, with trajectory I including children with the lowest values of BSA-IBW-adjusted CrCl and trajectory II including those with the highest values but, again, the difference was not statistically significant.

In multivariate linear regression models (Table III), adjusted for sex and children's current age, class of birthweight for gestational age, SBP z-score and class of BMI (3 categories – normal weight, overweight and obese), trajectory III was associated with a 2.68 to 3.75 lower eGFR, in the 2 models including the CysC-based formulas of Le Bricon and Filler, respectively, than in trajectory I. In addition, children in trajectory III also presented a 2.06 mL/min/1.73m^2 (95%CI -3.74 to -0.39, p=0.016) lower GFR, estimated by CKD-EPI, when compared to those in trajectory I.

DISCUSSION

In the present study we found that both Cr and CysC values were lower in children with slower and consistent weight gain (trajectory I) whilst eGFR was higher in these children. In contrast, children with persistent or during childhood weight gain (trajectories II and III) presented lower eGFR values. Additionally, prepubertal children with excessive weight gain during childhood (trajectory III) showed significantly lower values of GFR estimated by Filler, Le Bricon and CKD-EPI formulas, independently of children's current age and sex, class of birthweight for gestational age and class of BMI. We also found that, in a subsample of children, absolute CrCl values were lower in the group with consistent weight gain (trajectory I) whereas the highest values were found those with persistent excessive weight gain (trajectory II).

The relation of growth trajectories and kidney function during childhood has not yet been established. In fact, there are no previous studies addressing this specific issue. However, some studies already associated specific trajectories of growth with earlier pubertal maturation [5], with an increased risk of obesity, higher values of blood pressure, adiposity [6] and worse vascular profile [34] in childhood, and diabetes, hypertension and cardiovascular events [5] later in adulthood.

In which concerns to the association between obesity and renal damage, there is now strong evidence in adults that obesity is associated with an important increase in the incidence of chronic kidney disease [9,11,35,36] and, in the recent years, more and more studies have tried to ascertain if such an association exists in children. However, no studies have examined the impact of rapid weight gain, both in infancy and in childhood, in later kidney function. Many authors have reported inverse correlations between obesity or related anthropometric indexes and GFR in children [37,38,39], including a previous study of our group [12]. Nonetheless, there is still conflicting results in the literature, with some studies failing to achieve statistically significant differences in GFR values between obese and normal weight counterparts [40], others not reaching it after adjustment for metabolic factors [9] and others describing higher GFR values in obese children, possibly due to an initial phase of hyperfiltration, known to occur in the obesity-related kidney damage pathophysiology [41,42]. In the present study, we report that children in growth trajectories associated with increased weight gain presented lower eGFR, but higher absolute CrCl.

The described renal functional initial changes in the setting of obesity are glomerular hyperperfusion, hyperfiltration and later hypertension and progressive renal function decline [41,42]. At this age, we would expect to find higher eGFR values in obese children, what would be consistent with an initial phase of renal damage, but as discussed in a previous study of our group, the use of classic GFR formulas adjusted for BSA might introduce a bias, taking

into account the fact that BMI has a strong correlation with BSA [28]. Therefore, in this setting, GFR values adjusted to BSA are considerably underestimated in individuals with higher BMI, since an overadjustment is introduced in GFR estimation [43,44], which might falsely mask the initial phase of glomerular hyperfiltration [45,46]. IBW-based BSA has been identified as a promising option as an adjustment for GFR and has already been recognized as useful in other clinical areas, such as indexing ventricular mass in children [47]. A previous study of our group found that BSA-IBW-adjusted GFR values were significantly higher in overweight/obese children (as opposed to what happened when comparing BSA-adjusted GFR values which were lower in overweight/obese children) and performed a better correlation with absolute GFR values [28]. Our results seem to tend to be in line with these previous findings, since children in growth trajectories associated with obesity, specially those with persistent excessive weight gain, presented lower values of BSA-adjusted CrCl, but higher values of BSA-IBW-adjusted CrCl, but the differences were not statistically significant. In what concerns CrCl, we might not have achieved significant results due to a lack power, since fewer children were included in this analysis.

Many recent studies have addressed the issue of defining an accurate method to estimate GFR in children, either based on Cr or CysC, since most of the equations widely used were developed in children primarily with reduced GFR, which might lead to an underestimation of GFR in individuals without renal damage [48,49]. In addition, it is known that body composition can also influence the accuracy of eGFR equations. In obese adults, higher CysC levels were previously described in patients with higher BMI, leading to underestimation of GFR values when compared to exact exogenous GFR determinations [50,51]. Several studies highlight the importance of CysC as a filtration marker for GFR estimation in children [52,53]. However, other studies, have shown that the use of combined equations should be preferred, particularly in groups in which body composition might play a role [54], assuming that CysC based GFR values are underestimations in children with higher BMI [12]. In our study, in which trajectories with excessive weight gain were associated with lower values of eGFR, specially when GFR was estimated by CysC-based equations, we can hypothesize that these finding might be partially explained by the discussed limitations of CysC in the setting of obesity. Nonetheless, in the multivariate models, the decreased values of eGFR, estimated not only by the two CysC-based equations but also by the combined CKD-EPI equation, in trajectory III, persisted after adjustment for current BMI, which reinforces that, even taking into consideration intrinsic limitations of the methods of GFR estimation, children with weight gain during childhood already present altered renal function at the age of 8-9.

On the other hand, we reported that children in growth trajectories associated with obesity, particularly those with persistent excessive weight gain, presented higher absolute CrCl compared to those with slower and consistent weight gain. The increased absolute values found in the trajectories with more weight gain might be indicative that these children might in fact be in an initial phase of hyperfiltration caused by the excessive body mass. The analysis of these results increases our ability to withdraw more definitive conclusions on the direction of the association found between weight gain and GFR. But, regardless, of the direction of the aforementioned association, our results probably represent the first evidence on the early impact of rapid weight gain, both in infancy and in childhood, on the glomerular functioning of the young children's kidneys. In this context, our results of lower eGFR and higher absolute CrCl in children with increased weight gain can be explained by the fact that GFR estimated by both Cr and CysC equations and normalized to 1.73m^2 of body surface might underestimate GFR in overweight/obese children, in comparison with the absolute values of CrCl, which do not take body size into account.

In addition, weight and gestational age at birth may play an important effect upon later life GFR [55,56]. Since the number of nephrons is fixed at birth, children with intrauterine growth restriction, born small for gestational age or preterm and, therefore, with reduced nephron endowment, not only have an increased risk of obesity but are also more susceptible to suffer from chronic kidney disease in the future. Excessive weight gain, particularly during the rapid catch-up growth, promotes an increase in the metabolic and hemodynamic load of each individual nephron, leading to a stage of hyperfiltration, which is even more evident in individuals with reduced nephron mass [55]. In the present study, linear regression models to evaluate the effect of each trajectory on eGFR values were adjusted for classes of birthweight for gestational age, which confirmed that the GFR differences found were independent of this possible confounder.

The major strength of our study is the inclusion of a large and homogeneous sample of healthy prepubertal children, expected to be representative of their population, leading us to believe that our results can be generalized. In fact, we present a prospective analysis of a population-based cohort data from early life until mid-childhood with data on a previously not described association. The multiple weight measurements obtained from birth permitted a sensitive developmental perspective analysis of its impact on renal function, taking into account the weight "duration" and "intensity", and the age of onset. Moreover, we used growth mixture modeling which is considered an ideal flexible modeling approach to identify subpopulations with similar longitudinal trajectories. The analysis of our outcome one year later than the last exposure measurement obtained seems appropriate in a longitudinal approach. Additionally, we performed CysC and Cr measurements in a large sub-sample

of the cohort of more than 1000 children at the age of 7-9 years, allowing a GFR estimation based on both markers which, as previously discussed, has advantages when compared to single marker-based equations. The availability of detailed information regarding numerous pre- and postnatal variables enabled us to take into consideration the impact of potential confounders of the associations studied.

Nonetheless, some important limitations of our study have to be acknowledged. The analysis of renal function in a cross-sectional manner demands caution in causality interpretations. We intend to overcome this limitation by keeping regular evaluations of these children, with particular interest on their nutritional status and on the evolution of their renal function. Another important limitation is the fact that we could only assess renal function by 24-hour CrCl in 261 of the 1004 children enrolled in the study, which reinforces the need for future data collection to better ascertain the nature of the association between weight gain trajectories and renal function, not only during childhood but also later on during adulthood, evaluating the possibility of an increase in the incidence of chronic kidney disease and poorer renal survival in those adults that as children presented a worse growth profile. Another weakness of our study is that we only took into account a general measure (weight) and not fat distribution or other biochemical markers. The latter could allow more detailed analysis but we believe that this putative limitation was largely overcome by the amount of information, with thousands of weight records over time, which would be impossible to obtain for other markers.

Our study has led us to conclude that children with persistent or during childhood weight gain present significantly lower eGFR, when compared to children with slower and consistent weight gain. Our results support the influence of growth patterns in kidney function, which might be in line with other previously reported associations of excessive weight gain growth trajectories with higher risk of developing obesity and cardiovascular risk factors later in life [2,5,6]. Since the children included in our study are very young, still presenting renal function in the normal range, further work needs to be carried out to establish the clinical relevance of our findings. As the onset and development of obesity-associated renal disease is subtle, often remaining undetected for years, the widespread use of early markers, as NGAL and KIM-1, that have already shown good results as screening methods for early renal damage in obese children [9], might play an important role in the future in the early detection and prevention of renal damage in this setting.

Our results highlight the importance of prevention of obesity since early life. Future investigation focusing on growth patterns influence on cardiovascular and renal outcomes is crucial to help the early identification of at-risk children and to implement timely interventions to improve lifelong health.

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Table I. General baseline characteristics, including birth and current anthropometric data, of the study sample, by growth trajectories.

		Growth trajectory				
	All n = 1004	**I** n = 640	**II** n = 117	**III** n = 161	**IV** n = 86	p
Demographic and birth data						
Age (months)	89.99 ± 9.62	89.76 ± 9.50	89.21 ± 9.05	90.78 ± 10.15	91.24 ± 10.18	0.297
Male sex	510 (50.8%)	347 (54.2%)	64 (54.7%)	51 (31.7%)	48 (55.8%)	<0.001
Gestational age at birth (weeks)	38.82 ± 1.53	39.01 ± 1.19	38.97 ± 1.26	38.95 ± 1.26	37.03 ± 2.85	<0.001
Birthweight (g)	3232.7 ± 463.4	3262.1 ± 393.7	3474.4 ± 461.5	3190.7 ± 374.3	2768.2 ± 710.8	<0.001
Birthweight for gestational age[a]						
SGA	70 (7.0%)	41 (6.4%)	4 (3.4%)	13 (8.1%)	12 (14.0%)	
AGA	881 (87.9%)	570 (89.3%)	96 (82.1%)	145 (90.1%)	70 (81.4%)	<0.001
LGA	51 (5.1%)	27 (4.2%)	17 (14.5%)	3 (1.9%)	4 (4.7%)	
Anthropometric data						
Weight (kg)	28.05 ± 6.69	25.70 ± 4.93	35.64 ± 7.59	30.88 ± 6.60	29.97 ± 6.68	<0.001
z-score	0.79 ± 1.17	0.33 ± 0.94	2.28 ± 1.07	1.36 ± 0.88	1.15 ± 0.99	<0.001
Height (cm)	126.32 ± 6.99	124.67 ± 6.52	130.98 ± 7.00	128.32 ± 6.45	128.52 ± 6.93	<0.001
z-score	0.38 ± 0.95	0.09 ± 0.86	1.28 ± 0.95	0.69 ± 0.80	0.66 ± 0.83	<0.001
BMI (kg/m^2)	17.39 ± 2.74	16.42 ± 1.98	20.61 ± 3.12	18.62 ± 2.79	17.96 ± 2.59	<0.001
z-score	0.78 ± 1.19	0.36 ± 1.00	2.14 ± 1.08	1.31 ± 1.05	1.06 ± 1.08	<0.001
Classes of BMI[b]						
Normal weight	620 (61.8%)	485 (75.8%)	23 (19.7%)	68 (42.2%)	44 (51.2%)	
Overweight	224 (22.3%)	121 (18.9%)	28 (23.9%)	49 (30.4%)	26 (30.2%)	<0.001
Obese	160 (15.9%)	34 (5.3%)	66 (54.4%)	44 (27.3%)	16 (18.6%)	
Office SBP (mmHg)	104 ± 9	103 ± 9	108 ± 9	106 ± 9	104 ± 7	<0.001
z-score	0.02 ± 0.90	-0.13 ± 0.88	0.52 ± 0.83	0.24 ± 0.86	0.02 ± 0.88	<0.001
Office DBP (mmHg)	67 ± 8	67 ± 7	69 ± 9	68 ± 8	65 ± 8	0.011
z-score	0.52 ± 0.68	0.45 ± 0.65	0.85 ± 0.75	0.61 ± 0.67	0.40 ± 0.69	<0.001

The values presented are mean ± standard deviation or n (%), as appropriate.

SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[a] Children's classes of birthweight for gestational age were defined according to the Fenton growth charts [14].
[b] BMI z-scores classes were defined according to the World Health Organization classification [15].

Table II. Renal function markers and estimated glomerular filtration rates of the study sample by growth trajectories.

		Growth trajectory				
	All n = 1004	**I** n = 640	**II** n = 117	**III** n = 161	**IV** n = 86	**p**
Renal function markers						
Serum creatinine (mg/dL)	0.41 ± 0.06	0.41 ± 0.06	0.42 ± 0.06	0.42 ± 0.06	0.42 ± 0.06	0.024
Serum cystatin C (mg/L)	0.66 ± 0.07	0.65 ± 0.07	0.67 ± 0.06	0.67 ± 0.07	0.67 ± 0.07	0.003
Estimates of GFR						
GFR (Cr_Schwartz) (mL/min/1.73m^2)	129 ± 18	129 ± 18	131 ± 17	129 ± 18	128 ± 16	0.437
GFR (Cys_Filler) (mL/min/1.73m^2)	149 ± 18	150 ± 18	145 ± 15	146 ± 16	147 ± 18	0.002
GFR (Cys_LeBricon) (mL/min/1.73m^2)	124 ± 13	125 ± 13	122 ± 11	122 ± 12	123 ± 13	0.002
GFR (Cr-Cys_CKD-EPI) (mL/min/1.73m^2)	153 ± 10	154 ± 10	151 ± 9	152 ± 9	151 ± 9	0.005
GFR (Cr-Cys_Zappitelli) (mL/min/1.73m^2)	138 ± 16	139 ± 17	137 ± 15	136 ± 16	136 ± 15	0.107
GFR (Cr-Cys_Schwartz) (mL/min/1.73m^2)	111 ± 12	110 ± 12	111 ± 11	112 ± 12	110 ± 10	0.323
GFR (Cr-Cys_CKiD) (mL/min/1.73m^2)	111 ± 11	112 ± 11	112 ± 12	109 ± 11	111 ± 10	0.089
Absolute 24h-CrCl* (mL/min)	101 ± 24	98 ± 24	115 ± 23	104 ± 21	105 ± 24	0.005
BSA-adjusted 24h-CrCl* (mL/min/1.73m^2)	160 ± 33	162 ± 35	154 ± 24	155 ± 30	155 ± 32	0.341
BSA-IBW-adjusted CrCl* (mL/min/1.73m^2)	171 ± 36	169 ± 37	182 ± 30	174 ± 34	173 ± 36	0.370

The values presented are mean ± standard deviation.

* In the analysis of 24-hour CrCl only 261 children, with valid 24-hour urine samples, were included (165 in trajectory I, 25 in trajectory II, 45 in trajectory III, 26 in trajectory IV).

GFR, glomerular filtration rate; Cr, creatinine; Cys, cystatin C; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKiD, Chronic Kidney Disease in Children; CrCl, 24-hour creatinine clearance; BSA, body surface area calculated by Haycock equation; IBW, ideal body weight; BSA-IBW, body surface area calculated by Haycock equation using ideal body weight instead of child's real weight.

Table III. Mean differences in renal function markers and estimated glomerular filtration rates in the different growth trajectories, using trajectory I as reference.

	Growth trajectory			
	I	**II**	**III**	**IV**
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Estimates of GFR				
GFR (Cr_Schwartz) (mL/min/1.73m^2)	0	1.48 (-2.54;5.51) p=0.470	-0.04 (-3.34;3.27) p=0.983	-0.97 (-5.06;3.12) p=0.642
GFR (Cys_Filler) (mL/min/1.73m^2)	0	-2.87 (-6.78;1.04) p=0.150	-3.75 (-6.95;-0.54) p=0.022	-2.63 (-6.61;1.34) p=0.194
GFR (Cys_LeBricon) (mL/min/1.73m^2)	0	-2.05 (-4.86;0.76) p=0.152	-2.68 (-4.98;-0.38) p=0.023	-1.89 (-4.75;0.96) p=0.193
GFR (Cr-Cys_CKD-EPI) (mL/min/1.73m^2)	0	-1.81 (-3.85;0.24) p=0.083	-2.06 (-3.74;-0.39) p=0.016	-1.89 (-3.97;0.19) p=0.075
GFR (Cr-Cys_Zappitelli) (mL/min/1.73m^2)	0	-1.45 (-5.13;2.22) p=0.438	-2.51 (-5.52;0.51) p=0.103	-2.46 (-6.20;1.27) p=0.196
GFR (Cr-Cys_Schwartz) (mL/min/1.73m^2)	0	0.54 (-1.86;2.93) p=0.659	-0.53 (-2.49;1.44) p=0.598	-0.90 (-3.34;1.53) p=0.466
GFR (Cr-Cys_CKiD) (mL/min/1.73m^2)	0	0.59 (-1.81;2.99) p=0.629	-0.60 (-2.56;1.37) p=0.552	-0.91 (-3.35;1.52) p=0.463
Absolute 24h-CrCl* (mL/min)	0	3.93 (-6.21;14.07) p=0.446	0.33 (-7.73;8.39) p=0.936	-1.10 (-10.34;8.15) p=0.816
BSA-adjusted 24h-CrCl* (mL/min/1.73m^2)	0	-7.67 (-23.12;7.78) p=0.329	-3.63 (-15.91;8.66) p=0.562	-7.95 (-22.03;6.14) p=0.268
BSA-IBW-adjusted CrCl* (mL/min/1.73m^2)	0	-4.87 (-21.04;11.29) p=0.553	-2.41 (-15.26;10.44) p=0.712	-6.83 (-21.56;7.91) p=0.362

The values presented are linear regression coefficients (β) and 95% confidence intervals, estimated by multivariate linear regression models. The models were adjusted for children's current age in months, sex, children's class of birthweight for gestational age (according to the Fenton growth charts [14]), systolic blood pressure z-score and children's class of BMI (3 categories – normal weight, overweight and obese).

* In the analysis of 24-hour CrCl only 261 children, with valid 24-hour urine samples, were included (165 in trajectory I, 25 in trajectory II, 45 in trajectory III, 26 in trajectory IV).

GFR, glomerular filtration rate; Cr, creatinine; Cys, cystatin C; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKiD, Chronic Kidney Disease in Children; CrCl, 24-hour creatinine clearance; BSA, body surface area calculated by Haycock equation; IBW, ideal body weight; BSA-IBW, body surface area calculated by Haycock equation using ideal body weight instead of child's real weight.

Figure 1. Defined growth trajectories of weight in function of age in the Generation XXI cohort. The background grey lines represent the mean weight, mean minus 2 standard deviations and mean plus 2 standard deviations of the World Health Organization reference population.

Legend:
Trajectory I
Trajectory II
Trajectory III
Trajectory IV