



KIDNEYS UNDER PRESSURE IN CHILDREN

INTERPLAY BETWEEN OBESITY-RELATED COMORBIDITIES
AND LIFESTYLE CHANGES

Mark J.C.M. van Dam



KIDNEYS UNDER PRESSURE IN CHILDREN

INTERPLAY BETWEEN OBESITY-RELATED COMORBIDITIES AND LIFESTYLE CHANGES

Mark Jacob Carla Marion van Dam

Maastricht, 5 juni 2026

1. In the vast majority of children with overweight and obesity, serum creatinine (SCr)-based estimated glomerular filtration rate (eGFR) is within the age- and sex-specific reference range [this thesis]
2. In children with overweight and obesity, correlations between obesity-related comorbidities and SCr-based eGFR are highly dependent on the choice of eGFR equation [this thesis]
3. Based on the distribution of eGFR and absence of age- and sex-dependency, the SCr-based eGFR equations FAS-age, FAS-height and LMR18 should be used in children with overweight and obesity [this thesis]
4. Rescaled SCr adjusted for both sex and age (SCr/Q-age) and height (SCr/Q-height) provide a valid alternative to GFR-estimating equations [this thesis]
5. In longitudinal studies of children with overweight and obesity, GFR should not be adjusted for body surface area to avoid bias caused by changes in body composition rather than kidney function [this thesis]
6. Diagnosing obesity and appropriate management thereof should be integral to (pediatric) nephrology care [this thesis – valorisation]
7. Since SCr values in children are prone to misinterpretation, clinical laboratory reports should include eGFR (preferably FAS-age) for children aged 2 years and older [personal opinion]
8. Of all the paths you take in life, a few of them should be dirt [adapted from John Muir]

KIDNEYS UNDER PRESSURE IN CHILDREN
INTERPLAY BETWEEN OBESITY-RELATED COMORBIDITIES
AND LIFESTYLE CHANGES

Mark Jacob Carla Marion van Dam

© Mark J.C.M. van Dam, Maastricht, 2026

All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, without prior written permission of the author or, when applicable, of the publishers of the publications.

Cover design: Marisya van Dam – Janssen

Layout and production: ProefschriftMaken | proefschriftmaken.nl

ISBN: 978-94-6534-351-8

The research presented in this thesis was conducted within the Institute NUTRIM for Nutrition and Translational Research in Metabolism and is based on data from the Centre for Overweight Adolescent and Children's Healthcare (COACH) Maastricht.

Financial support from Maastricht University Medical Centre+ for the publication of this thesis is gratefully acknowledged.

KIDNEYS UNDER PRESSURE IN CHILDREN
INTERPLAY BETWEEN OBESITY-RELATED COMORBIDITIES
AND LIFESTYLE CHANGES

Proefschrift

voor het behalen van de graad van Doctor aan de Universiteit Maastricht,
onder gezag van Rector Magnificus, prof. dr. Jan Smits,
overeenkomstig met het besluit van het College van Decanen,
te verdedigen in het openbaar
op vrijdag 5 juni 2026 om 10.00 uur

door

Mark Jacob Carla Marion van Dam

Promotores:

Prof. dr. A.C.E. Vreugdenhil (Universiteit Maastricht/Maastricht UMC+)

Em. Prof. dr. H. Pottel (KU Leuven Campus Kortrijk, België)

Beoordelingscommissie:

Voorzitter:

Prof. dr. M.E. Rubio Gozalbo (Universiteit Maastricht/Maastricht UMC+)

Leden:

Dr. A. Bökenkamp (Amsterdam UMC)

Prof. dr. D. Mekahli (KU Leuven, België)

Prof. dr. E.G.A.H. van Mil (Universiteit Maastricht)

TABLE OF CONTENTS

Chapter 1.	General introduction	7
Chapter 2.	Creatinine-based GFR-estimating equations in children with overweight and obesity	31
Chapter 3.	Relation between obesity-related comorbidities and kidney function estimation in children	59
Chapter 4.	Blood pressure in children with overweight and obesity: associations with estimated kidney function and metabolic aberrations	83
Chapter 5.	Real-time continuous glucose monitoring and estimated kidney function in children with overweight and obesity	101
Chapter 6.	The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity	123
Chapter 7.	The evaluation of kidney function estimation during lifestyle intervention in children with overweight and obesity	141
Chapter 8.	General discussion	157
Addendum	Summary	189
	Samenvatting	195
	Valorisation	201
	Dankwoord	207
	About the author	215
	List of publications	217

1

General introduction

BACKGROUND

Obesity is a chronic complex disease defined by excessive fat deposits that can impair health. It is generally accepted that obesity is harmful for children (1). In 2022, the global prevalence of overweight was estimated to be 37 million children under the age of 5 years and over 390 million children in the 5 to 19 years age category (2). Among children aged 5 to 19 years, about 8% of children were living with obesity (160 million children) and the prevalence of overweight and obesity in this age category has risen dramatically from 8% in 1990 to 20% in 2022 (2). In the Netherlands, in 2023, 8.6% and 4.1% of the children between 4 and 17 years suffered from overweight and obesity respectively (3). Multiple obesity-related comorbidities, such as hypertension and glucose dysregulation, are frequently present in children (4). Moreover, obesity and its related conditions during childhood are likely to track into adulthood (5). Therefore, the current childhood obesity pandemic leads to many health-related issues for future adults. Although the knowledge of obesity-related health issues has increased during the last decades, some obesity-related comorbidities are less known to healthcare providers. This thesis focuses on the relation between childhood overweight and obesity and kidney disease.

Since the first report describing a relation between severe obesity and kidney disease in 1974 (6), it has become widely accepted that obesity is harmful for the kidneys. The World Kidney Day in 2017 was dedicated to the harmful consequences of obesity and its association with kidney disease and the attention to this topic has increased ever since. Chronic kidney disease (CKD) and obesity-related comorbidities have in common that they aggravate under the surface, and obesity-related CKD is referred to as a 'hidden consequence' of the obesity pandemic (7). CKD is defined as abnormalities of kidney structure or function, present for a minimum of 3 months and with implications for health (8). CKD already is a major public health challenge, with a global prevalence estimated around 10% (9) which is highly likely to increase as a consequence of the obesity pandemic (7). The exact prevalence of children with CKD, both globally and in the Netherlands, is not known. One of the reasons for this is that kidney disease reveals itself in most cases after many years, and loss of nephrons and thus decline in kidney function happens often undetected in the early stage of kidney disease. It is therefore of major importance to identify conditions, including modifiable risk factors such as obesity, hypertension and proteinuria, that increase the risk for future kidney disease (10). This helps clinicians make an early diagnosis and initiate early nephron-protective strategies, such as lifestyle modification, and facilitate monitoring for complications.

Obesity is harmful for the kidneys in multiple ways. First, obesity is known to worsen the kidney outcome in patients with primary kidney diseases. Moreover, obesity itself has negative effects on the kidneys. Finally, obesity-related comorbidities such as hypertension and diabetes are well-known causes for the development and progression

of CKD. All of these pathways through which obesity adversely affects the kidneys will be described in the following sections.

Obesity is known to aggravate primary kidney diseases, both congenital and acquired, and is therefore an important modifiable risk factor. For example, obesity is one of the major prognostic factors for an adverse kidney outcome in children with a solitary functioning kidney (11). An important finding, since a solitary functioning kidney is a common condition, affecting congenitally about one in 1400 children (12). Moreover, in young adults with autosomal dominant polycystic kidney disease (ADPKD), obesity is independently associated with estimated glomerular filtration rate (eGFR) decline in an early disease stage (13, 14). Finally, in patients with IgA nephropathy, the world's most common glomerulonephritis, patients with obesity have a lower eGFR compared to lean patients (15). Finally, data from the pediatric transplant population suggest that obesity of the transplant recipient may be associated with accelerated graft loss (16).

Besides the role of obesity in patients with primary kidney disease, obesity per se, so independent from well-known obesity-related consequences such as hypertension and diabetes, is a risk factor for the development and progression of CKD in patients with no underlying kidney disease (17). Moreover, obesity-related glomerulopathy is a distinct proteinuric kidney disease, histologically defined as glomerulomegaly and focal segmental glomerulosclerosis. The exact prevalence of this condition, however, is unclear since it can only be diagnosed by means of a kidney biopsy (18, 19). In a retrospective cohort study among 239 adults with morbid obesity, about 30% of the patients had biopsy-proven obesity-related glomerulopathy, alone or in combination with other kidney disease (20).

There is evidence that obesity during childhood is associated with CKD later in life (10, 21). In a longitudinal analysis of the Bogalusa Heart Study cohort, a higher BMI in childhood was more common in young adults who developed end-stage kidney disease (22). In a study among 1.2 million Israelis (prior to their military conscription), overweight and obesity at the age of 17 years were associated with all-cause treated end-stage kidney disease (Hazard ratio (HR) 3.00, 95% confidence interval (CI): 2.50-3.60, and HR 6.89, 95% CI: 5.52-8.59, respectively) (23). In another well-designed case-control study among Swedish males, overweight and obesity at the age of around 18 years, were associated with increased risk of end-stage kidney disease (Odds ratio (OR) 2.36 (95% CI: 1.79-3.13) and OR 3.53 (95% CI: 2.04-6.11), for overweight and obesity respectively) compared to males with a BMI between 18.5 and 25 kg/m² (24). Finally, there is the Medical Research Council National Survey of Health and Development study (25, 26): 5362 singleton children born in 1946 in England, Scotland and Wales were systematically evaluated with repeated measurements of BMI between the age of 2 and 20 years. Children were classified according to the following overweight classes: 'never', 'pre-pubertal

only', 'pubertal onset' or 'always'. This was related to the presence of CKD at the age of 60-64 years (creatinine- or cystatin C-based eGFR < 60 ml/min/1.73m² or urine albumin-creatinine ratio (ACR) ≥ 3.5 mg/mmol). Relative to being in the 'never' overweight class, being in the 'pubertal onset' or 'always' overweight class was associated with CKD at the age of 60-64 years, more pronounced for cystatin C-based eGFR (OR 2.04, 95% CI 1.09-3.82) than for creatinine-based eGFR (OR 1.27, 95% CI 0.71-2.29) and urine ACR (OR 1.33, 95% CI 0.70-2.54). Adjustment for important confounders such as socioeconomic status and smoking had little impact on the effect estimates.

All these studies underline the importance of an adequate approach to childhood obesity in order to possibly prevent or delay progression of CKD in adulthood.

PATHOPHYSIOLOGY OF OBESITY-RELATED KIDNEY DISEASE

How childhood obesity exactly leads to CKD later in life is not known, which is not surprising since studies are complicated by multiple confounders and the decades of follow-up required to detect associations. However, from studies conducted in animals and adults, there is growing evidence of how obesity affects the kidneys. The hallmark in the etiology of obesity-related kidney disease is glomerular hyperfiltration, a state of supraphysiological single-nephron glomerular filtration rate (GFR). Glomerular hyperfiltration is associated with loss of kidney function (27) and with all-cause mortality and adverse cardiovascular outcomes (28, 29). Glomerular hyperfiltration can be 'absolute' or 'relative', depending on whether there is a normal or reduced number of functioning nephrons. Independent of 'absolute' or 'relative' hyperfiltration, a persistent increase in single-nephron GFR eventually leads to hyperfiltration injury via the mechanism first hypothesized by Brenner et al. in 1996 (30): glomerular hyperfiltration is associated with glomerular hypertension that leads, via podocyte detachment, to albuminuria/proteinuria, glomerulosclerosis and eventually decline in kidney function (31, 32).

In clinical setting, a high GFR (so whole-kidney GFR, the sum of the single-nephron GFR) is often used as a surrogate marker for single-nephron glomerular hyperfiltration. It is important to consider that this surrogate marker is not perfect but inevitable, since determining single-nephron GFR and nephron mass in routine clinical setting is still impossible (33). In human donor studies though, single-nephron GFR (calculated as whole-kidney GFR divided by the number of nephrons) is correlated with obesity (34). Arguably, a whole-kidney GFR greater than 135 ml/min/1.73m² might be reliably used as a cut-off value (35), although other values have been suggested as well (36). The impossibility to measure single-nephron GFR in clinical setting and the controversy about

the definition of glomerular hyperfiltration based on whole-kidney GFR, among others, results in an unclear prevalence of this condition both in children and in adults.

While glomerular hyperfiltration can be physiological after high-protein meal consumption or during pregnancy (37), in prolonged state, glomerular hyperfiltration is thought to play an important role in the initiation of CKD in various conditions such as diabetes mellitus (38, 39), polycystic kidney disease (40), solitary functioning kidney (12), sickle cell disease (41) and obesity (42, 43). In Figure 1.1, a schematic representation of the pathway in which absolute glomerular hyperfiltration results in chronic kidney disease is shown. A persistently increased single-nephron GFR (and thus whole-kidney GFR in the presence of a normal number of functioning nephrons) leads to albuminuria, glomerulosclerosis and a decline in kidney function and thus CKD.

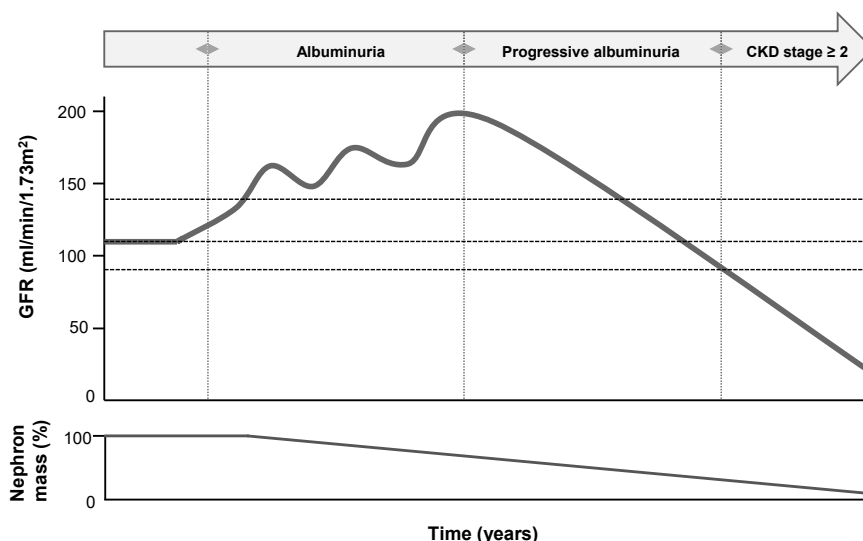


Figure 1.1. Schematic representation of the pathway in which absolute glomerular hyperfiltration results in chronic kidney disease. The three dotted lines represent GFR 90 ml/min/1.73m², 107.3 ml/min/1.73m² and 135 ml/min/1.73m², corresponding to the cut-off GFR for CKD stage ≥ 2 , the average GFR in children from the age of 2 years, and a commonly used cut-off value for the definition of glomerular hyperfiltration, respectively. A persistently increased single-nephron GFR (and thus whole-kidney GFR in the presence of a normal number of functioning nephrons) leads to microalbuminuria, albuminuria, glomerulosclerosis, and a decline in kidney function. Over time, the functional nephron mass decreases. This figure is based on Cortinovis et al. (31) and Tonneijck et al. (38).

So why do kidneys hyperfiltrate in the context of obesity? Based mainly on experimental data, two central hypotheses have been suggested to explain this (18). First, the hemodynamic hypothesis states that glomerular hyperfiltration is the result of an imbalance of vasoactive mediators that regulate the tone of the afferent and efferent arterioles. In individuals with obesity, vasodilation of the afferent arterioles and vasoconstriction of efferent arterioles lead to higher GFR, renal plasma flow, and filtration fraction (18, 44). Second, the tubulocentric hypothesis states that hyperfiltration is enhanced by an increase in tubular sodium reabsorption in the proximal tubule via sodium-glucose co-transporter 2 (SGLT2) and 1 (SGLT1). This in turn leads to decreased sodium concentration at the macula densa and thus deactivation of the tubuloglomerular feedback system. This leads to reduction in afferent arteriole resistance resulting in increased glomerular perfusion. The causes of increased sodium reabsorption in obesity are multiple and include increased activity of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system, and compression of the kidney parenchyma by visceral, perirenal and kidney sinus fat (18, 31).

Renin-angiotensin-aldosterone system (RAAS)

The RAAS is traditionally recognized for its role in regulating blood pressure, fluid balance, and electrolyte homeostasis. In addition to these functions, it plays a key role in various other (patho)physiological processes, including tissue growth and hypertrophy, inflammation, and the interference with glucose and lipid metabolism, among others (45).

In individuals with obesity, the RAAS is chronically overactive (18, 44, 45). In human adipose tissue and in primary cultured adipocytes, as well as in animal models, levels of RAAS components like angiotensinogen, renin, angiotensin-converting enzyme (ACE), aldosterone, and angiotensin II type 1 and type 2 receptors are increased (45, 46). This local RAAS in adipose tissue plays an important role in modulating lipogenesis, lipolysis, adipogenesis, and systemic and adipose tissue inflammation (46, 47).

Blockade of the RAAS is highly effective in delaying the progression of CKD in children and adults (48-51). Furthermore, a post hoc analysis of the REIN trial demonstrated that the antiproteinuric effect of the ACE inhibitor ramipril was significantly greater in individuals with overweight and obesity compared with individuals with a normal BMI. Moreover, treatment with ramipril reduced the incidence of end-stage kidney disease among patients with obesity by 86%, compared with reductions of 45% and 42% among those with overweight and normal weight, respectively (52).

Taken together, these findings suggest that overactivation of the RAAS in obesity leads to excessive sodium reabsorption, thereby contributing to the development of

hypertension. Therefore, RAAS inhibition remains a cornerstone in the management of obesity-related hypertension.

Whilst the pathogenesis of obesity-related kidney disease is incompletely understood, we will next discuss important obesity-related alterations that contribute to the development of kidney disease.

Hypertension

Arterial hypertension is one of the most common causes of CKD and cardiovascular disease worldwide, and thus a major global health issue. Childhood hypertension has long been considered an uncommon condition, mostly secondary to kidney, renovascular, endocrine, or cardiac disorders. This situation has changed over the last few decades, since primary hypertension can present as early as 3 years of age and is nowadays the predominant form of hypertension in children older than 6 years (53). Elevated blood pressure in childhood tracks into adulthood (54). The increased prevalence of primary hypertension in children is directly related to the increased prevalence of childhood obesity, and hypertension might be the main comorbidity in children with overweight and obesity (55). The estimated prevalence of primary hypertension in children with overweight and obesity has been found to be 20 to 40%, depending on different reference values and the degree of adiposity (56).

Not all children with primary hypertension have adiposity and not all children with overweight or obesity have hypertension (57). Therefore, the question is whether primary hypertension and obesity-related hypertension are the same disease. The pathophysiology of both entities is complex and incompletely understood, but for obesity-related hypertension, disturbed body composition and distribution of fat mass, increased sodium reabsorption (as described previously) and metabolic aberrations, such as hyperinsulinemia and hyperuricemia, seem to play a role (57, 58).

Glucose dysregulation

Traditionally, type 2 diabetes mellitus (T2DM) was considered to be an adult-onset disease resulting from long-term glucose dysregulation and failure of pancreatic β cells to produce insulin. With the childhood obesity pandemic, the prevalence of T2DM in children has increased with 95% over the past 16 years and was estimated to be 0.67 per 1000 children aged 10 to 19 years in 2017 in the United States of America (59). Compared with the onset of T2DM in adulthood, adults with onset of T2DM in childhood have a higher incidence of kidney disease. In the TODAY2 study, the cumulative incidence of diabetic kidney disease was about 50% mean 13.3 ± 1.8 years after the diagnosis of T2DM (60). This underlines the importance of addressing obesity and glucose dysregulation during childhood.

Whilst T2DM itself is relatively rare in children, precursors of T2DM, such as insulin resistance (IR) and impaired glucose tolerance (IGT), are more common in children with overweight and obesity. The prevalence of IR is estimated to be up to 72% in children with adiposity, largely depending on the methods and definitions used (61). Hyperglycemia and IR are important risk factors for obesity- and diabetes-related kidney disease (18, 38, 62). In a hyperglycemic state, the glucose concentration in the ultrafiltrate in the proximal tubule increases, which induces the expression of the sodium-glucose cotransporter 2 (SGLT2) and enhances reabsorption of both sodium and glucose. The enhanced sodium reabsorption causes lower sodium concentrations in the distal tubule which deactivates the tubuloglomerular feedback mechanism, and as a result from dilatation of the afferent glomerular arterioles and increased renin secretion, GFR increases. In this manner, hyperglycemia leads to single-nephron glomerular hyperfiltration (18, 38, 62).

Also in the absence of T2DM, hyperglycemic glucose excursions are common in children with obesity (63, 64). However, to the best of our knowledge, no studies exist on the relation between kidney function and (high) glucose levels in free-living conditions in children without diabetes.

Altered lipid metabolism and ectopic lipid in the kidney ('fatty kidneys')

An altered lipid metabolism is suggested to play an important role in the pathogenesis of obesity-related kidney disease. Impaired expandability of adipose tissue and increased sensitivity of visceral adipose tissue to lipolysis might lead to increased concentrations of nonesterified fatty acids (also known as free fatty acids) into the circulation, resulting in deposition in non-adipose tissues such as the liver and the kidney (65, 66). Triacylglyceride accumulation in the human kidney cortex correlates with BMI (67). The ectopic lipid in the kidney, referred to as 'fatty kidneys', is associated with structural and functional changes of mesangial cells, podocytes, and proximal tubular cells (68). The archetypical example of ectopic lipid, non-alcoholic fatty liver disease (NAFLD), is independently associated with CKD as well (69). Although non-invasive assessment of 'fatty kidneys' in clinical setting is still challenging, the presence and distribution of ectopic lipid in the kidney may be a promising biomarker of obesity-related kidney disease (68).

WEIGHT LOSS AND OBESITY-RELATED KIDNEY DISEASE

Since obesity aggravates the outcome of kidney diseases with different etiologies, weight loss represents a logical therapeutic option. Several studies demonstrate that obesity-related kidney disease and/or glomerular hyperfiltration might be reversible in case of weight loss. In adults with obesity without overt kidney disease, bariatric surgery resulted in a reduction of high GFR (suggesting improvement of glomerular hyperfiltration), and

albuminuria/proteinuria (70-75). In 34 patients with obesity and T2DM with preserved kidney function, a 6-month calorie-restricted diet was associated with a significant reduction in measured GFR (mGFR), suggesting improvement of hyperfiltration (76). In patients with CKD, weight loss following bariatric surgery or lifestyle intervention results in a reduction in proteinuria (77, 78). There was no consistency in the effects of weight loss on kidney function, which might be explained by differences in the methods used to assess kidney function.

Studies on the effect of weight loss on kidney function in children with overweight and obesity are scarce. To the best of our knowledge, there are no studies focusing on lifestyle intervention and kidney function in children with obesity without overt kidney disease. A prospective multicentre study among 242 adolescents demonstrated a reduction in albuminuria and an increase in eGFR following bariatric surgery (79, 80). Note that in these studies eGFR was indexed for body surface area (BSA) (unit: ml/min/1.73m²) and not de-indexed for BSA (unit: ml/min). The effect of bariatric surgery on eGFR therefore might rather be the result of changes in BSA rather than an effect on absolute GFR.

ASSESSMENT OF GLOMERULAR FILTRATION RATE (GFR) IN CHILDREN

Glomerular filtration rate (GFR) is the standard measure of kidney function and is critical for the diagnosis and management of kidney diseases (81). GFR describes the amount of fluid passage across the glomerular membrane per minute, and is expressed in milliliters per minute. Since GFR is strongly dependent on the blood flow through the renal arteries, increased cardiac output is associated with increased GFR, and therefore absolute GFR (unit: ml/min) is higher in adults than in children. To correct for this difference in body proportion, GFR is often indexed for BSA, an iconic convention within nephrology with its origin in 1928 (82) that is not without discussion nowadays; GFR is expressed in milliliters per minute and then multiplied by 1.73 and divided by BSA (in m²) (83). Of note, 1.73m² was considered to be the BSA of the average 25-year-old American at the beginning of the 20th century, nowadays an average BSA around 2.0 m² would be probably more appropriate (83).

From the 5th week of pregnancy kidneys start to develop and nephron formation is achieved by the 35-36th week of pregnancy, on average about one million per kidney with a high correlation with birth weight. While the kidneys do grow in size after birth, no new nephrons are formed. During life, nephron mass decreases which causes a natural decline in kidney function. Children with a lower nephron mass, such as children with a congenital solitary functioning kidney or children born premature or small for gestational age, or children with higher nephron degradation, will sooner 'outgrow' their kidneys and

will progress to CKD earlier. In the first days and weeks after birth, GFR rapidly increases from about 20 ml/min/1.73m² followed by a more gradual increase in the weeks and months thereafter (84, 85). Indexation of GFR for BSA shows that children reach 'adult' GFR values by about 2 years of age (86, 87).

True GFR is impossible to measure since the filtration process simultaneously takes place in millions of glomeruli. As the best alternative method in clinical setting ('gold standard'), GFR is measured using the clearance of exogenous markers. While many exogenous markers have been used (such as technetium-99m diethylenetriaminepentaacetic acid (99mTc-DTPA), inulin, iothalamate, chromium-51 ethylenediaminetetraacetic acid (51Cr-EDTA)) (88), iothexol has become the most commonly used method over recent years (89). GFR measurements can be performed using two different procedures: the renal clearance procedure, involving blood and urine samples and the plasma clearance procedure, involving only blood samples (83). Independent of the method, determination of mGFR necessitates intravenous injection of the exogenous marker and due to this invasive nature, mGFR has a minor role in clinical practice in (pediatric) nephrology. As an alternative, GFR can be measured by the clearance of creatinine, in which serum creatinine (SCr) concentrations are related to timed urinary creatinine excretion (most commonly: 24-hour excretion). While (pediatric) nephrologist still use this method, it has been proven insufficiently accurate in clinical setting and should therefore be abandoned (90, 91).

Instead, in clinical practice, the preferred method is to estimate GFR based on serum concentrations of endogenous markers. To be ideal, an endogenous marker should have a constant production rate; no hindrance to passage across the glomerular filtration barrier; no protein binding; no extra-renal metabolism or tubular excretion or reabsorption; and accurate measurement assay (92). Although the ideal endogenous marker does not seem to exist, several markers have been shown to fulfil many of these property criteria, such as SCr and cystatin C (92). At this moment, SCr is still the most commonly used endogenous biomarker and the marker of first choice for GFR estimation both in children and adults.

Creatinine, a small molecule of 113 Da, originates from the creatine/phosphocreatine pathway. Creatine is synthesized in the liver and kidneys and is stored predominantly in striated muscle cells where it, via phosphocreatine, phosphorylates ADP into ATP when energy demand is high. Both phosphocreatine and creatine spontaneously degrade to creatinine (93). Besides this, SCr concentrations are dependent on dietary intake of cooked meat and fish and creatine supplements. The volume of distribution of creatinine is total body water (94), and SCr is heavily influenced by muscle mass (95). SCr may not increase until the GFR has decreased by about 50%. Creatinine can be measured in multiple ways. The least expensive Jaffe reaction method (96) has been replaced by the enzymatic method in most laboratories. Until the implementation of isotope dilution

mass spectroscopy (IDMS)-based calibration of creatinine measurement in the first decade of the 21st century, reference values varied between hospitals. Since then, the use of the IDMS-based standard has allowed the establishment of uniform reference ranges over the whole age spectrum (97, 98).

In children, normal values for SCr are highly age-dependent (97, 98), as displayed in Figure 1.2.

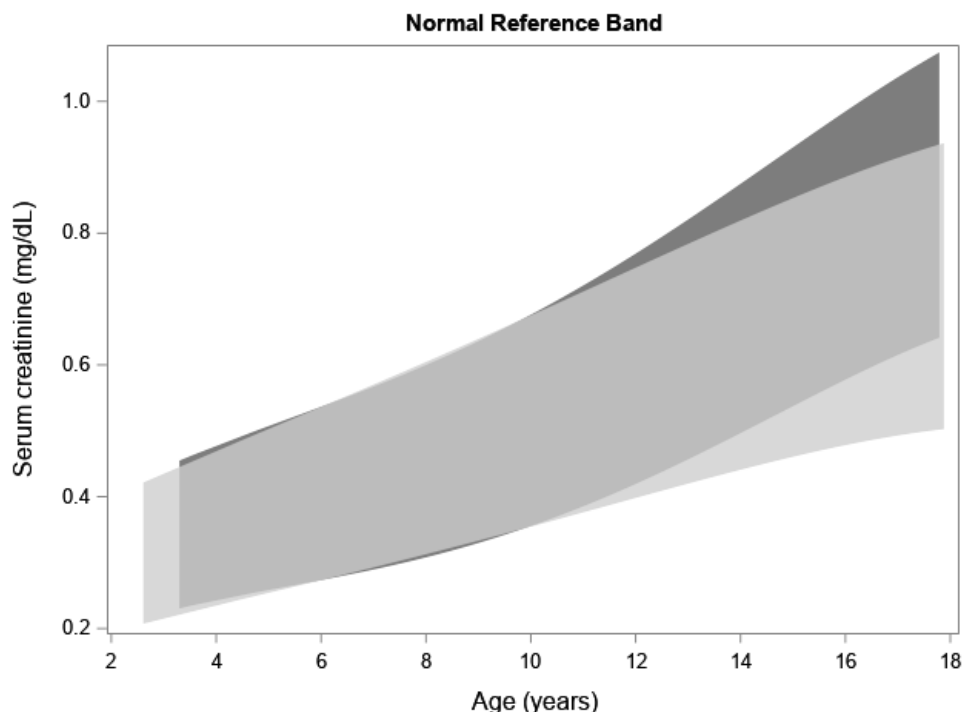


Figure 1.2. Serum creatinine (SCr, in mg/dL) against age for females (light grey) and males (dark grey). The band represents the reference interval for SCr based on the Hoste polynomials (99).

Neonates have relatively high SCr levels in the first week of life, reflecting maternal SCr levels due to diaplacental exchange of creatinine (100). SCr then drops with the lowest normal values found at about 2 months of age. From then on SCr levels rise steadily as a result of increasing muscle mass. Between the age of 2 and 14 years SCr increases linearly with age without any differences between males and females. From the age of 14 years, sex-differences arise and SCr levels off to constant values of 0.70 mg/dL (about 62 $\mu\text{mol/L}$) for adult females, and 0.90 mg/dL (about 80 $\mu\text{mol/L}$) for adult males.

The concept of GFR-estimating equations

GFR-estimating equations are developed using the correlation between mGFR and the inverse of endogenous marker concentrations, anthropometric data and other covariates that can influence marker concentrations, such as sex, age and height (101). Correction for these confounders reduces the risk of inaccuracy (bias) and imprecision (random error). Since possibly not all confounding factors are known, an eGFR equation thus reflects the population used for its generation which should be considered when using a specific equation for a specific population and/or patient. In other words, the eGFR value from equations based on data from healthy children will therefore probably differ from those from equations based on data from children with a kidney disease (83, 92). Besides, the calibration of the endogenous marker assays in the laboratory should be checked and the choice of equation should be adapted to this; which is the case for all modern pediatric eGFR equations as they are all developed using IDMS equivalent SCr assays (mostly enzymatic assays). Moreover, for correct estimation, the endogenous marker must be in a steady state. In case of rapid declining or improving GFR, the equilibrium between the blood compartment and distribution volume of the endogenous marker shifts, resulting in over- and underestimation of eGFR, respectively (102). Finally, one must consider that the eGFR value is an estimate of the mGFR. Even when using the best-performing, and thus most commonly used equations, only about 90% and 40% of the estimates fall within $\pm 30\%$ and $\pm 10\%$ of mGFR values (103, 104).

The population used for generating the equation differs between the Chronic Kidney Disease in Children (CKiD) consortium, and Pottel's group and the European Kidney Function Consortium (EKFC). The CKiD consortium equations were generated from the CKiD cohort: a multicentre, prospective cohort study of children with CKD from the United States of America and Canada. The most recent CKiD equation (CKiD-U25 (105)), was generated from data from 1764 visits of 618 children (63% males, median age 13 years, median height percentile around 30, median mGFR around 46-49 ml/min/1.73m²) and validated against a population about half this size (n=310) with similar characteristics. In contrast, the equations by Pottel's group used data from 2056 children in the development dataset, 695 children in the internal validation dataset, and 1254 children in an external validation dataset (106). These datasets included children with eGFR in the normal reference range as well as children with reduced eGFR (106).

Besides a different population, the CKiD consortium and the group of Pottel have chosen a completely different approach in order to correct for age- and sex-dependent differences in creatinine synthesis. The CKiD equations use height as a surrogate marker for muscle mass, and a coefficient k to reflect age- and sex-related differences in muscle mass. Whilst multiple CKiD equations exist, most clinicians use the "bedside Schwartz equation" (107). The improved CKiD-U25 equation (105), with variable sex- and age-specific k values up to the age of 25 years, overcomes the implausible age-dependent decline (108). The

approach by Pottel and colleagues is often referred to as “Full Age Spectrum (FAS)”, since the same method is used in children and adults. The main concept is based on the fact that the average GFR value of 107.3 ml/min/1.73m² for healthy children from the age of 2 years (which is independent of age), corresponds to the average SCr concentration. Since the average SCr values are heavily age-dependent, SCr values of an individual child are ‘rescaled’ (or ‘normalized’) using the median SCr value of a child with corresponding age (and sex in case of an age >14 years; so-called, Q-age), resulting in SCr/Q (109). Hoste and colleagues extended the equation and introduced Q-height (the median SCr values in children with corresponding height instead of age), in order to perform better in children older than 14 years (99).

AIMS OF THE THESIS

The major aims of this thesis were:

- 1 To gain more insight into kidney function in children with overweight and obesity by:
 - a Investigating SCr and the most commonly used SCr-based GFR-estimating equations in children with overweight and obesity without overt kidney disease;
 - b Investigating whether rescaled SCr for sex and age, or height (SCr/Q-age and SCr/Q-height, respectively), can be used as an alternative or add-on marker for GFR estimation in children with overweight and obesity;
- 2 To gain more insight into the relation between eGFR and obesity-related comorbidities by examining if and how SCr, SCr/Q and SCr-based eGFR are correlated with anthropometric parameters and obesity-related comorbidities such as hypertension, glucose dysregulation, dyslipidemia, hyperuricemia, and sleep-disordered breathing;
- 3 To gain more insight into the effect of a multidisciplinary lifestyle intervention program on SCr, SCr/Q and GFR estimation in children with overweight and obesity.

Figure 1.3 provides an overview of these aims. A more detailed description of the pathophysiology of obesity-related kidney disease can be found in several review articles (18, 19, 44, 62, 110-113).

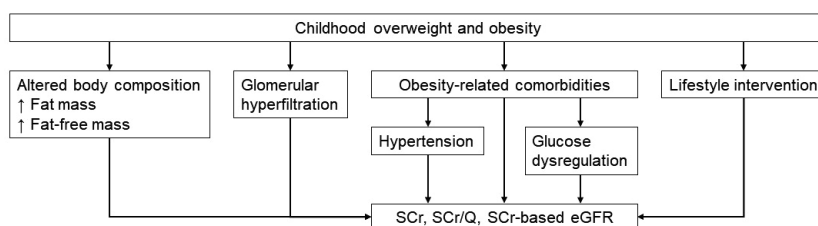


Figure 1.3. Schematic overview of the aims of this thesis.

OUTLINE OF THE THESIS

For this thesis, data were used from the Centre of Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre + (MUMC+) MosaKids Children's Hospital. In this expertise centre for children and adolescents with overweight and obesity, a multidisciplinary lifestyle intervention program was developed. Since the COACH program is and has been an ongoing program, the cohorts can differ between studies.

With the increasing prevalence of childhood obesity and related development of CKD, there is a critical need to understand how best to assess kidney function in children with obesity, both concerning research and clinical questions such as CKD classification and drug dosing. Therefore, we conducted a study on SCr, rescaled SCr for age and sex (SCr/Q-age), and the most commonly used SCr-based GFR-estimating equations in children with overweight and obesity, described in **Chapter 2**.

Clinical studies examining associations between obesity-related comorbidities and kidney function in children have yielded inconsistent results (21). We postulate that these discrepant results might be, at least partially, caused by bias and random error in the used GFR-estimating equations. Therefore, we conducted a study, described in **Chapter 3**, in which we compared different SCr-based GFR-estimating equations and their correlations with anthropometric variables and obesity-related comorbidities. In this study we examined as well whether rescaled SCr for sex and age (SCr/Q-age) or height (SCr/Q-height) might be used as an alternative for GFR-estimating equations.

In **Chapter 4**, we describe a study on blood pressure (BP) in children with overweight and obesity. In childhood, the increased prevalence of obesity is directly related to the increased prevalence of hypertension. Hypertension is one of the most important potentially reversible causes of cardiovascular and kidney disease worldwide. Although the exact pathophysiology of obesity-related hypertension is not known, there seems to be a major overlap with the pathophysiology of obesity-related CKD. Therefore, in this chapter, we examined the anthropometric and metabolic characteristics of children

within different office BP categories and investigated whether office BP and eGFR are correlated.

In **Chapter 5**, we describe a study on glycemic variables in children with overweight and obesity. As described earlier, insulin resistance and hyperglycemia are common findings in children with obesity and important risk factors for obesity- and diabetes related kidney disease. To the best of our knowledge, no studies exist on the relation between eGFR and (high) glucose levels in free-living conditions in children without diabetes. Therefore, we conducted a cross-sectional study in children with overweight and obesity to examine this correlation, in which the glycemic profile was evaluated with both oral glucose tolerance test and using real-time continuous glucose monitoring.

Our last two studies are longitudinal. Studies on the effect of weight loss on eGFR in children with overweight and obesity are scarce. Moreover, to the best of our knowledge, there are no studies focusing on lifestyle intervention and eGFR in children with obesity without overt kidney disease. Therefore we conducted a study, described in **Chapter 6**, in which we examined the effect of one year lifestyle intervention on eGFR.

With the increasing prevalence of childhood obesity, and increasing attention and possibilities to adjust lifestyle, more and more children with overweight and obesity are in a situation that includes alterations of body proportions, especially when lifestyle adjustment includes increased physical activity. Since SCr levels are heavily influenced by muscle mass, it is highly likely that SCr levels change in response to lifestyle adjustment. However, at this moment it is unclear how this influences GFR estimation. Therefore, we conducted a study, described in **Chapter 7**, in which we examined the course of SCr, SCr/Q and eGFR during the COACH lifestyle intervention.

Finally, **Chapter 8** provides a general discussion including the main findings from the studies, methodological considerations, clinical implications and future perspectives.

REFERENCES

1. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med*. 2017;377(1):13-27
2. Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *Lancet*. 2024;403(10431):1027-50
3. National Institute of Public Health and Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM). Informatie over Volksgezondheid en Zorg. Overgewicht - Jongeren. 2021 [cited 2022 December 14]. Available from: <https://www.vzinfo.nl/overgewicht/leeftijd-en-geslacht-jongeren>
4. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic Risks and Severity of Obesity in Children and Young Adults. *N Engl J Med*. 2015;373(14):1307-17
5. Juhola J, Magnussen CG, Viikari JS, Kähönen M, Hutri-Kähönen N, Jula A, et al. Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. *J Pediatr*. 2011;159(4):584-90
6. Weisinger JR, Kempson RL, Eldridge FL, Swenson RS. The nephrotic syndrome: a complication of massive obesity. *Ann Intern Med*. 1974;81(4):440-7
7. Kovesdy CP, Furth SL, Zoccali C. Obesity and kidney disease: hidden consequences of the epidemic. *Pediatr Nephrol*. 2017;32(4):537-45
8. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int*. 2024;105(4s):S117-s314
9. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. *Lancet*. 2017;389(10075):1238-52
10. Stern-Zimmer M, Calderon-Margalit R, Skorecki K, Vivante A. Childhood risk factors for adulthood chronic kidney disease. *Pediatr Nephrol*. 2021;36(6):1387-96
11. Groen In 't Woud S, Roeleveld N, Westland R, Renkema KY, Steffens MG, Gracchi V, et al. Uncovering risk factors for kidney injury in children with a solitary functioning kidney. *Kidney Int*. 2023 Jan;103(1):156-165
12. Schreuder MF. Life with one kidney. *Pediatr Nephrol*. 2018 Apr;33(4):595-604
13. Nowak KL, You Z, Gitomer B, Brosnahan G, Torres VE, Chapman AB, et al. Overweight and Obesity Are Predictors of Progression in Early Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol*. 2018;29(2):571-8
14. Dachy A, Decuypere JP, Vennekens R, Jouret F, Mekahli D. Is autosomal dominant polycystic kidney disease an early sweet disease? *Pediatr Nephrol*. 2022;37(9):1945-55
15. Kanbay M, Yildiz AB, Yavuz F, Covic A, Ortiz A, Siriopol D. The role of body mass index on IgA nephropathy prognosis: a systematic review and meta-analysis. *Int Urol Nephrol*. 2022 Oct;54(10):2567-2579
16. Ladhani M, Lade S, Alexander SI, Baur LA, Clayton PA, McDonald S, et al. Obesity in pediatric kidney transplant recipients and the risks of acute rejection, graft loss and death. *Pediatr Nephrol*. 2017;32(8):1443-50
17. Garofalo C, Borrelli S, Minutolo R, Chiodini P, De Nicola L, Conte G. A systematic review and meta-analysis suggests obesity predicts onset of chronic kidney disease in the general population. *Kidney Int*. 2017;91(5):1224-35
18. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol*. 2016;12(8):453-71

19. Martínez-Montoro JI, Morales E, Cornejo-Pareja I, Tinahones FJ, Fernández-García JC. Obesity-related glomerulopathy: Current approaches and future perspectives. *Obes Rev.* 2022;23(7):e13450
20. Choung HG, Bombback AS, Stokes MB, Santoriello D, Campenot ES, Batal I, et al. The spectrum of kidney biopsy findings in patients with morbid obesity. *Kidney Int.* 2019;95(3):647-54
21. Jadresic L, Silverwood RJ, Kinra S, Nitsch D. Can childhood obesity influence later chronic kidney disease? *Pediatr Nephrol.* 2019;34(12):2457-77
22. Muntner P, Arshad A, Morse SA, Patel DA, Manapatra PD, Reisin E, et al. End-stage renal disease in young black males in a black-white population: longitudinal analysis of the Bogalusa Heart Study. *BMC Nephrol.* 2009 Dec 2;10:40
23. Vivante A, Golan E, Tzur D, Leiba A, Tirosh A, Skorecki K, et al. Body mass index in 1.2 million adolescents and risk for end-stage renal disease. *Arch Intern Med.* 2012;172(21):1644-50
24. Sundin PO, Udumyan R, Sjöström P, Montgomery S. Predictors in adolescence of ESRD in middle-aged men. *Am J Kidney Dis.* 2014;64(5):723-9
25. Silverwood RJ, Pierce M, Thomas C, Hardy R, Ferro C, Sattar N, et al. Association between younger age when first overweight and increased risk for CKD. *J Am Soc Nephrol.* 2013;24(5):813-21
26. Silverwood RJ, Pierce M, Hardy R, Thomas C, Ferro C, Savage C, et al. Early-life overweight trajectory and CKD in the 1946 British birth cohort study. *Am J Kidney Dis.* 2013;62(2):276-84
27. Cachat F, Combescure C, Cauderay M, Girardin E, Chehade H. A systematic review of glomerular hyperfiltration assessment and definition in the medical literature. *Clin J Am Soc Nephrol.* 2015;10(3):382-9
28. Park M, Yoon E, Lim YH, Kim H, Choi J, Yoon HJ. Renal hyperfiltration as a novel marker of all-cause mortality. *J Am Soc Nephrol.* 2015;26(6):1426-33
29. Kanbay M, Ertuglu LA, Afsar B, Ozdogan E, Kucuksumer ZS, Ortiz A, et al. Renal hyperfiltration defined by high estimated glomerular filtration rate: A risk factor for cardiovascular disease and mortality. *Diabetes Obes Metab.* 2019;21(11):2368-83
30. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int.* 1996;49(6):1774-7
31. Cortinovis M, Perico N, Ruggenenti P, Remuzzi A, Remuzzi G. Glomerular hyperfiltration. *Nat Rev Nephrol.* 2022;18(7):435-51
32. Kanbay M, Copur S, Bakir CN, Covic A, Ortiz A, Tuttle KR. Glomerular hyperfiltration as a therapeutic target for CKD. *Nephrol Dial Transplant.* 2024;39(8):1228-38
33. Denic A, Glasscock RJ, Rule AD. Single-Nephron Glomerular Filtration Rate in Healthy Adults. *N Engl J Med.* 2017;377(12):1203-4
34. Denic A, Mathew J, Lerman LO, Lieske JC, Larson JJ, Alexander MP, et al. Single-Nephron Glomerular Filtration Rate in Healthy Adults. *N Engl J Med.* 2017;376(24):2349-57
35. Pottel H, Adebayo OC, Nkoy AB, Delanaye P. Glomerular hyperfiltration: part 1 - defining the threshold - is the sky the limit? *Pediatr Nephrol.* 2023 Aug;38(8):2523-2527
36. Chakkerla HA, Denic A, Kremers WK, Stegall MD, Larson JJ, Ravipati H, et al. Comparison of high glomerular filtration rate thresholds for identifying hyperfiltration. *Nephrol Dial Transplant.* 2020;35(6):1017-26
37. Helal I, Fick-Brosnahan GM, Reed-Gitomer B, Schrier RW. Glomerular hyperfiltration: definitions, mechanisms and clinical implications. *Nat Rev Nephrol.* 2012;8(5):293-300
38. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *J Am Soc Nephrol.* 2017;28(4):1023-39

39. Molitch ME, Gao X, Bebu I, de Boer IH, Lachin J, Paterson A, et al. Early Glomerular Hyperfiltration and Long-Term Kidney Outcomes in Type 1 Diabetes: The DCCT/EDIC Experience. *Clin J Am Soc Nephrol*. 2019;14(6):854-61
40. Helal I, Reed B, McFann K, Yan XD, Fick-Brosnahan GM, Cadnapaphornchai M, et al. Glomerular hyperfiltration and renal progression in children with autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2011;6(10):2439-43
41. Haymann JP, Stankovic K, Levy P, Avellino V, Tharaux PL, Letavernier E, et al. Glomerular hyperfiltration in adult sickle cell anemia: a frequent hemolysis associated feature. *Clin J Am Soc Nephrol*. 2010;5(5):756-61
42. Chagnac A, Herman M, Zingerman B, Erman A, Rozen-Zvi B, Hirsh J, et al. Obesity-induced glomerular hyperfiltration: its involvement in the pathogenesis of tubular sodium reabsorption. *Nephrol Dial Transplant*. 2008;23(12):3946-52
43. Adebayo OC, Nkoy AB, van den Heuvel LP, Labarque V, Levtschenko E, Delanaye P, et al. Glomerular hyperfiltration: part 2-clinical significance in children. *Pediatr Nephrol*. 2023 Aug;38(8):2529-2547
44. Yau K, Kuah R, Cherney DZI, Lam TKT. Obesity and the kidney: mechanistic links and therapeutic advances. *Nat Rev Endocrinol*. 2024;20(6):321-35
45. Ruster C, Wolf G. The role of the renin-angiotensin-aldosterone system in obesity-related renal diseases. *Semin Nephrol*. 2013;33(1):44-53
46. Cassis LA, Police SB, Yiannikouris F, Thatcher SE. Local adipose tissue renin-angiotensin system. *Curr Hypertens Rep*. 2008;10(2):93-8
47. Saiki A, Ohira M, Endo K, Koide N, Oyama T, Murano T, et al. Circulating angiotensin II is associated with body fat accumulation and insulin resistance in obese subjects with type 2 diabetes mellitus. *Metabolism*. 2009;58(5):708-13
48. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med*. 1993;329(20):1456-62
49. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med*. 2001;345(12):861-9
50. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). *Lancet*. 1997;349(9069):1857-63
51. Wühl E, Trivelli A, Picca S, Litwin M, Peco-Antic A, Zurowska A, et al. Strict blood-pressure control and progression of renal failure in children. *N Engl J Med*. 2009;361(17):1639-50
52. Mallamaci F, Ruggenenti P, Perna A, Leonardi D, Tripepi R, Tripepi G, et al. ACE inhibition is renoprotective among obese patients with proteinuria. *J Am Soc Nephrol*. 2011;22(6):1122-8
53. Gupta-Malhotra M, Banker A, Shete S, Hashmi SS, Tyson JE, Barratt MS, et al. Essential hypertension vs. secondary hypertension among children. *Am J Hypertens*. 2015;28(1):73-80
54. Azegami T, Uchida K, Tokumura M, Mori M. Blood Pressure Tracking From Childhood to Adulthood. *Front Pediatr*. 2021;9:785356
55. Flechtner-Mors M, Thamm M, Wiegand S, Reinehr T, Schwab KO, Kiess W, et al. Comorbidities related to BMI category in children and adolescents: German/Austrian/Swiss Obesity Register APV compared to the German KiGGS Study. *Horm Res Paediatr*. 2012;77(1):19-26
56. Flechtner-Mors M, Neuhauser H, Reinehr T, Roost HP, Wiegand S, Siegfried W, et al. Blood pressure in 57,915 pediatric patients who are overweight or obese based on five reference systems. *Am J Cardiol*. 2015;115(11):1587-94

57. Litwin M, Kułaga Z. Obesity, metabolic syndrome, and primary hypertension. *Pediatr Nephrol.* 2021;36(4):825-37
58. Wühl E. Hypertension in childhood obesity. *Acta Paediatr.* 2019;108(1):37-43
59. Lawrence JM, Divers J, Isom S, Saydah S, Imperatore G, Pihoker C, et al. Trends in Prevalence of Type 1 and Type 2 Diabetes in Children and Adolescents in the US, 2001-2017. *JAMA.* 2021;326(8):717-27
60. Bjornstad P, Drews KL, Caprio S, Gubitosi-Klug R, Nathan DM, Tesfaldet B, et al. Long-Term Complications in Youth-Onset Type 2 Diabetes. *N Engl J Med.* 2021;385(5):416-26
61. van der Aa MP, Knibbe CA, Boer A, van der Vorst MM. Definition of insulin resistance affects prevalence rate in pediatric patients: a systematic review and call for consensus. *J Pediatr Endocrinol Metab.* 2017;30(2):123-31
62. Sawyer A, Zeitler E, Trachtman H, Bjornstad P. Kidney Considerations in Pediatric Obesity. *Curr Obes Rep.* 2023;12(3):332-44
63. Rijks J, Karnebeek K, van Dijk JW, Dorenbos E, Gerver WJ, Stouthart P, et al. Glycaemic Profiles of Children With Overweight and Obesity in Free-living Conditions in Association With Cardiometabolic Risk. *Sci Rep.* 2016;6:31892
64. Hegedus E, Salvy SJ, Wee CP, Naguib M, Raymond JK, Fox DS, et al. Use of continuous glucose monitoring in obesity research: A scoping review. *Obes Res Clin Pract.* 2021;15(5):431-8.
65. Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature.* 2006;444(7121):881-7
66. Gai Z, Wang T, Visentin M, Kullak-Ublick GA, Fu X, Wang Z. Lipid Accumulation and Chronic Kidney Disease. *Nutrients.* 2019;11(4)
67. Bobulescu IA, Lotan Y, Zhang J, Rosenthal TR, Rogers JT, Adams-Huet B, et al. Triglycerides in the human kidney cortex: relationship with body size. *PloS One.* 2014;9(8):e101285
68. de Vries AP, Ruggerenti P, Ruan XZ, Praga M, Cruzado JM, Bajema IM, et al. Fatty kidney: emerging role of ectopic lipid in obesity-related renal disease. *Lancet Diabetes Endocrinol.* 2014;2(5):417-26
69. Musso G, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, et al. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. *PLoS Med.* 2014;11(7):e1001680
70. Chagnac A, Weinstein T, Herman M, Hirsh J, Gaftor U, Ori Y. The effects of weight loss on renal function in patients with severe obesity. *J Am Soc Nephrol.* 2003;14(6):1480-6
71. Friedman AN, Moe S, Fadel WF, Inman M, Mattar SG, Shihabi Z, et al. Predicting the glomerular filtration rate in bariatric surgery patients. *Am J Nephrol.* 2014;39(1):8-15
72. Lieske JC, Collazo-Clavell ML, Sarr MG, Rule AD, Bergstralh EJ, Kumar R. Gastric bypass surgery and measured and estimated GFR in women. *Am J Kidney Dis.* 2014;64(4):663-5
73. von Scholten BJ, Persson F, Svane MS, Hansen TW, Madsbad S, Rossing P. Effect of large weight reductions on measured and estimated kidney function. *BMC Nephrol.* 2017;18(1):52
74. Navarro-Díaz M, Serra A, Romero R, Bonet J, Bayés B, Homs M, et al. Effect of drastic weight loss after bariatric surgery on renal parameters in extremely obese patients: long-term follow-up. *J Am Soc Nephrol.* 2006;17(12 Suppl 3):S213-7
75. Serra A, Granada ML, Romero R, Bayés B, Cantón A, Bonet J, et al. The effect of bariatric surgery on adipocytokines, renal parameters and other cardiovascular risk factors in severe and very severe obesity: 1-year follow-up. *Clin Nutr.* 2006;25(3):400-8
76. Ruggerenti P, Abbate M, Ruggiero B, Rota S, Trillini M, Aparicio C, et al. Renal and Systemic Effects of Calorie Restriction in Patients With Type 2 Diabetes With Abdominal Obesity: A Randomized Controlled Trial. *Diabetes.* 2017;66(1):75-86

77. Afshinnia F, Wilt TJ, Duval S, Esmaeili A, Ibrahim HN. Weight loss and proteinuria: systematic review of clinical trials and comparative cohorts. *Nephrol Dial Transplant*. 2010;25(4):1173-83
78. Li K, Zou J, Ye Z, Di J, Han X, Zhang H, et al. Effects of Bariatric Surgery on Renal Function in Obese Patients: A Systematic Review and Meta Analysis. *PloS One*. 2016;11(10):e0163907
79. Nehus EJ, Khoury JC, Inge TH, Xiao N, Jenkins TM, Moxey-Mims MM, et al. Kidney outcomes three years after bariatric surgery in severely obese adolescents. *Kidney Int*. 2017;91(2):451-8
80. Bjornstad P, Nehus E, Jenkins T, Mitsnefes M, Moxey-Mims M, Dixon JB, et al. Five-year kidney outcomes of bariatric surgery differ in severely obese adolescents and adults with and without type 2 diabetes. *Kidney Int*. 2020;97(5):995-1005
81. Gaspari F, Perico N, Remuzzi G. Measurement of glomerular filtration rate. *Kidney Int Suppl*. 1997;63:S151-4
82. McIntosh JF, Möller E, Van Slyke DD. Studies of urea excretion. III: The Influence of Body Size on Urea Output. *J Clin Invest*. 1928;6(3):467-83
83. Pottel H. Measuring and estimating glomerular filtration rate in children. *Pediatr Nephrol*. 2017;32(2):249-63
84. Smeets NJL, Int'Hout J, van der Burgh MJP, Schwartz GJ, Schreuder MF, de Wildt SN. Maturation of GFR in Term-Born Neonates: An Individual Participant Data Meta-Analysis. *J Am Soc Nephrol*. 2022;33(7):1277-92
85. Piepsz A, Tondeur M, Ham H. Revisiting normal (51)Cr-ethylenediaminetetraacetic acid clearance values in children. *Eur J Nucl Med Mol Imaging*. 2006;33(12):1477-82
86. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. *Clin J Am Soc Nephrol*. 2009;4(11):1832-43
87. Pottel H, Mottaghy FM, Zaman Z, Martens F. On the relationship between glomerular filtration rate and serum creatinine in children. *Pediatr Nephrol*. 2010;25(5):927-34
88. Ebert N, Bevc S, Bökenkamp A, Gaillard F, Hornum M, Jager KJ, et al. Assessment of kidney function: clinical indications for measured GFR. *Clin Kidney J*. 2021;14(8):1861-70
89. Delanaye P, Ebert N, Melsom T, Gaspari F, Mariat C, Cavalier E, et al. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: How to measure glomerular filtration rate with iohexol? *Clin Kidney J*. 2016;9(5):682-99
90. Herget-Rosenthal S, Bökenkamp A, Hofmann W. How to estimate GFR-serum creatinine, serum cystatin C or equations? *Clin Biochem*. 2007;40(3-4):153-61
91. Soveri I, Berg UB, Björk J, Elinder CG, Grubb A, Mejare I, et al. Measuring GFR: a systematic review. *Am J Kidney Dis*. 2014;64(3):411-24
92. den Bakker E, Gemke R, Bokenkamp A. Endogenous markers for kidney function in children: a review. *Crit Rev Clin Lab Sci*. 2018;55(3):163-83
93. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev*. 2000;80(3):1107-213
94. Schloerb PR. Total body water distribution of creatinine and urea in nephrectomized dogs. *Am J Physiol*. 1960;199:661-5
95. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol*. 2008;3(2):348-54
96. Husdan H, Rapoport A. Estimation of creatinine by the Jaffe reaction. A comparison of three methods. *Clin Chem*. 1968;14(3):222-38
97. Pottel H, Vrydaghs N, Mahieu B, Vandewynckele E, Croes K, Martens F. Establishing age/sex related serum creatinine reference intervals from hospital laboratory data based on different statistical methods. *Clin Chim Acta*. 2008;396(1-2):49-55

98. Ceriotti F, Boyd JC, Klein G, Henny J, Queraltó J, Kairisto V, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem.* 2008;54(3):559-66
99. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aïssa A, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant.* 2014;29(5):1082-91
100. Hodari AA, Mariona FG, Houlihan RT, Peralta J. Creatinine transport in the maternal-fetal complex. *Obstet Gynecol.* 1973;41(1):47-55
101. den Bakker E, Bökenkamp A, Haffner D. Assessment of Kidney Function in Children. *Pediatr Clin North Am.* 2022;69(6):1017-1035
102. Slort PR, Ozden N, Pape L, Offner G, Tromp WF, Wilhelm AJ, et al. Comparing cystatin C and creatinine in the diagnosis of pediatric acute renal allograft dysfunction. *Pediatr Nephrol.* 2012;27(5):843-9
103. Pottel H, Delanaye P, Schaeffner E, Dubourg L, Eriksen BO, Melsom T, et al. Estimating glomerular filtration rate for the full age spectrum from serum creatinine and cystatin C. *Nephrol Dial Transplant.* 2017;32(3):497-507
104. Björk J, Nyman U, Berg U, Delanaye P, Dubourg L, Goffin K, et al. Validation of standardized creatinine and cystatin C GFR estimating equations in a large multicentre European cohort of children. *Pediatr Nephrol.* 2019;34(6):1087-98
105. Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ. Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. *Kidney Int.* 2021;99(4):948-56
106. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, et al. Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate : A Cross-sectional Analysis of Pooled Data. *Ann Intern Med.* 2021;174(2):183-91
107. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol.* 2009;20(3):629-37
108. Pottel H, Björk J, Delanaye P, Nyman U. Evaluation of the creatinine-based chronic kidney disease in children (under 25 years) equation in healthy children and adolescents. *Pediatr Nephrol.* 2022;37(9):2213-6
109. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol.* 2012;27(6):973-9
110. Nehus E, Mitsnefes M. Kidney consequences of obesity. *Pediatr Nephrol.* 2025;40(6):1879-93
111. Kounatidis D, Vallianou NG, Stratigou T, Voukali M, Karampela I, Dalamaga M. The Kidney in Obesity: Current Evidence, Perspectives and Controversies. *Curr Obes Rep.* 2024;13(4):680-702
112. Mangat G, Nair N, Barat O, Abboud B, Pais P, Bagga S, et al. Obesity-related glomerulopathy in children: connecting pathophysiology to clinical care. *Clin Kidney J.* 2023;16(4):611-8
113. Câmara NO, Iseki K, Kramer H, Liu ZH, Sharma K. Kidney disease and obesity: epidemiology, mechanisms and treatment. *Nat Rev Nephrol.* 2017;13(3):181-90

2

Creatinine-based GFR-estimating equations in children with overweight and obesity

Mark J.C.M. van Dam, Hans Pottel, Anita C.E. Vreugdenhil

*Pediatr Nephrol.*2022 Oct;37(10):2393-2403

ABSTRACT

Background. With the increasing prevalence of childhood obesity and related development of chronic kidney disease (CKD), there is a critical need to understand how best to assess kidney function in children with obesity. Since serum creatinine (SCr) is recommended as marker of first choice for GFR estimation, we evaluated and compared creatinine-based GFR equations in children with overweight and obesity.

Methods. 600 children with overweight and obesity (53.5% female; mean age 12.20 ± 3.28 years; mean BMI z-score 3.31 ± 0.75) were included from the Centre for Overweight Adolescent and Children's Healthcare (COACH).

Results. Serum creatinine (SCr), normalized using Q-age polynomials obtained from reference values, results in median and mean SCr/Q value close to '1' for all age-groups, and 96.5% of the children have a SCr/Q within the reference band [0.67 – 1.33], corresponding to the 2.5th and 97.5th percentile. eGFR-CKiD (bedside Schwartz equation) and Schwartz-Lyon decreased with age, whereas eGFR-EKFC and modified CKD-EPI40 showed no age-dependency, but the distribution of eGFR-values was not symmetrical. eGFR-CKiD Under 25 (CKiDU25) demonstrated no age-dependency but major sex differences were observed. eGFR-FAS-age, FAS-height and adjusted-creatinine revised Lund-Malmö (LMR18) showed a relatively symmetrical distribution and no age-dependency.

Conclusions. Serum creatinine (SCr) values of children with overweight and obesity are mostly within the reference range for children. Normalization of SCr using reference Q-age polynomials works very well in this cohort. After evaluation of the different equations, we suggest that FAS-age, FAS-height and LMR18 are the preferred creatinine-based GFR-estimating equations in children with overweight and obesity.

INTRODUCTION

Glomerular filtration rate (GFR) estimation is essential for daily practice in (pediatric) nephrology, since direct measurement of GFR is still considered to be too invasive for routine clinical use. Estimated GFR (eGFR) equations are highly dependent on the data used for the development of the equation and incorrect use of an equation might lead to severe inaccuracy (1). In adults with overweight and obesity, GFR estimating equations fail in their precision to estimate GFR (2). Given the increasing prevalence of childhood obesity, there is a need to obtain more knowledge on how best to estimate GFR in this population, both concerning research and clinical questions such as drug dosing or chronic kidney disease (CKD) classification.

GFR-estimating equations using endogenous filtration markers (such as serum creatinine (SCr) and cystatin C) are suffering from inaccuracy (bias) and imprecision (random error). When using these equations physicians should be aware of the factors besides the GFR that influence serum concentrations of endogenous filtrations markers (the 'non-GFR determinants') such as dietary intake, synthesis, tubular absorption and secretion, and kidney-independent elimination (3). In order to partly correct for these non-GFR determinants demographic and clinical variables such as age, sex, height and body surface area (BSA) are implemented in eGFR equations. Though, these factors do not fully encompass the altered body proportions in children and adults with adiposity. Moreover, most commonly used eGFR equations were developed and validated in individuals with predominantly normal body composition (4).

Although other endogenous filtration markers are available, SCr is still recommended as marker of first choice for GFR evaluation. Creatinine, synthesized in the liver and kidneys, is stored predominantly in striated muscle cells (5). The volume of distribution of creatinine is total body water (6) and it is known that SCr is influenced by lean body mass (7). Besides endogenous creatinine productions, dietary protein intake and creatine supplements may contribute to the serum creatinine concentrations. (3) In children, SCr linearly increases with age between the age of 2 and 14 years and levels off to constant values of 0.90 mg/dL for adult males and 0.70 mg/dL for adult females. GFR is indexed with BSA, allowing comparison among children and adults (1, 8-11). Albeit indexation of GFR for BSA for individuals with altered body proportions is a controversial topic (2, 12, 13), almost all current creatinine-based GFR estimating equations have been designed for GFR indexed with BSA (1, 4).

The association between obesity in adulthood and incident CKD and CKD progression is currently well established (14) and there is some evidence for an association between childhood obesity and CKD later in life (15). This, combined with the increasing prevalence of childhood obesity, underlines the need to understand how best to assess kidney

function in children with obesity. Therefore, we evaluated SCr and creatinine-based GFR-estimating equations in children with overweight and obesity.

METHODS

Setting and study inclusion

For this study, baseline data were used from participants of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre + (MUMC+). COACH is a multidisciplinary lifestyle program in which the health status of children with overweight and obesity is evaluated, as described previously (16). Children who enter the COACH program are evaluated thoroughly in order to examine possible secondary causes of overweight and obesity and obesity-associated comorbidities. This first evaluation includes history taking, clinical examination and laboratory tests among others. All 662 participants who entered the COACH program between January 1st 2011 and April 1st 2019 were considered for inclusion in this study. Children with secondary causes of overweight, a history of (congenital or acquired) kidney disease that was known before inclusion into our program, diabetes mellitus and/or current use of antihypertensive medication were excluded from this study (n=13). Moreover, in 39 children the value for serum creatinine (SCr) was missing. 5 children did not meet the criteria for overweight or obesity and 5 children were older than 18 years and were thus excluded from this study. Finally, 600 children were included in this study. The study met the guidelines administered by the Declaration of Helsinki and was approved by the Medical Ethical Committee of the MUMC+. Informed consent was obtained from all parents or legal guardian, and children, provided they were 12 years or older.

Clinical assessment and anthropometry

All children underwent physical examination. Weight and height were determined using a digital scale (Seca, Chino, CA) and digital stadiometer (De Grood Metaaltechniek, Nijmegen, the Netherlands) respectively. The International Obesity Task Force (IOTF) criteria were used to define overweight, obesity and severe obesity (17). Body surface area (BSA) was calculated using height (cm) and weight (kg), using the Haycock et al. equation: $(Ht) (BSA = 0.024265 \times Wt^{0.5378} \times Ht^{0.3964})$ (18).

Creatinine-based GFR-estimating equations

Serum creatinine (SCr) was measured using the enzymatic method (Cobas 8000, Roche), equivalent to the isotope dilution mass spectrometry (IDMS) gold standard method. Creatinine references values (median SCr for 'healthy children', so-called Q-values) were obtained from the literature (19, 20). These Q-values were used to calculate SCr/Q ('normalized SCr') which is supposed to be age- and sex-independent for healthy subjects. It has been shown by Pottel et al. (21) that the reference band for SCr/Q in children is

defined by [0.67 – 1.33], with lower and upper limit corresponding to the 2.5th and 97.5th percentile. GFR was estimated using the following creatinine-based GFR-estimating equations, displayed in Table 2.1:

- Height-independent full-age spectrum equation, referred to as FAS-age (22);
- Height-dependent full-age spectrum equation, referred to as FAS-height (20);
- The new European Kidney Function Consortium (EKFC) equation, referred to as EKFC (23);
- Updated bedside Schwartz or CKiD equation (24);
- Schwartz-Lyon equation (25);
- CKiD under 25 years equation (CKiDU25) (26);
- Adjusted-creatinine revised Lund-Malmö equation, referred to as LMR18 (27);
- CKD-EPI equation with age-adjusted creatinine values, referred to as CKD-EPI40 (28);
- Improving renal complications in Adolescents with T2D through Research equation, referred to as iCARE (29).

Table 2.1. Overview of the creatinine-based GFR estimating equations examined in this study.

FAS-age (22)	$eGFR = 107.3 / (SCr / Q_{age})$, in which $Q_{age} = 0.21 + 0.057 \times Age - 0.0075 \times Age^2 + 0.00064 \times Age^3 - 0.000016 \times Age^4$ for males and $Q_{age} = 0.23 + 0.034 \times Age - 0.0018 \times Age^2 + 0.00017 \times Age^3 - 0.0000051 \times Age^4$ for females.
FAS-height (20)	$eGFR = 107.3 / (SCr / Q_{height})$, in which $Q_{height} = 3.94 - 13.4 \times L + 17.6 \times L^2 - 9.84 \times L^3 + 2.04 \times L^4$, in which L represents height in meters for males and females.
EKFC (23)	$eGFR = 107.3 / \left(SCr / Q \right)^A \left[\times 0.990^{Age-40} \text{ if } age > 40 \text{ years} \right]$ with A = +0.322 when $SCr / Q < 1$ and A = +1.132 when $SCr / Q \geq 1$, in which Q (in $\mu\text{mol/L}$) is obtained from $LN(Q) = 3.200 + 0.259 \times Age - 0.543 \times LN(Age) - 0.00763 \times Age^2 + 0.0000790 \times Age^3$ for males and $LN(Q) = 3.080 + 0.177 \times Age - 0.223 \times LN(Age) - 0.00596 \times Age^2 + 0.0000686 \times Age^3$ for females, when $2 \leq Age \leq 25$ years.
CKiD (‘bedside Schwartz’) (24)	$eGFR = k \times L / SCr$ in which k is 0.413, L represents height in cm and SCr is expressed in mg/dL.
Schwartz- Lyon (25)	$eGFR = k \times L / SCr$ in which k is 0.413 if males age > 13 years and k is 0.368 otherwise, L represents height in cm and SCr is expressed in mg/dL.
CKiDU25 (26)	$eGFR = k \times L / SCr$ in which for males $k = 39.0 \times 1.008^{(age-12)}$ for $1 \leq age < 12$, $k = 39.0 \times 1.045^{(age-12)}$ for $12 < age < 18$, and $k = 50.8$ for $18 \leq age < 25$; for females $k = 36.1 \times 1.008^{(age-12)}$ for $1 \leq age < 12$, $k = 36.1 \times 1.023^{(age-12)}$ for $12 < age < 18$, and $k = 41.4$ for $18 \leq age < 25$. L represents height in meters and SCr is expressed in mg/dL.

- LMR18 (27) $eGFR = e^{X - 0.0158 \times \max(\text{Age}; 18) + 0.438 \times \ln(\max(\text{Age}; 18))}$ in which $X = 2.50 + 0.0121 \times (150 - Q)$ for females with $Q < 150 \mu\text{mol/L}$, $X = 2.50 - 0.926 \times \ln(Q/150)$ for females with $Q \geq 150 \mu\text{mol/L}$, $X = 2.56 + 0.00968 \times (180 - Q)$ for males with $Q < 180 \mu\text{mol/L}$, and $X = 2.56 - 0.926 \times \ln(Q/180)$ for males with $Q \geq 180 \mu\text{mol/L}$, in which Q is obtained from $LN(Q) = LN(SCr) + 0.259 \times (18 - \text{Age}) - 0.543 \times \ln^{18}/\text{Age} - 0.00763 \times (18^2 - \text{Age}^2) + 0.0000790 \times (18^3 - \text{Age}^3)$ in males; and $LN(Q) = LN(SCr) + 0.177 \times (18 - \text{Age}) - 0.223 \times \ln^{18}/\text{Age} - 0.00596 \times (18^2 - \text{Age}^2) + 0.0000686 \times (18^3 - \text{Age}^3)$; $\max(\text{Age}; 18)$ represents the maximum of Age (actual age of the patient) and 18 years (the applied age threshold).
- CKD-EPI40 (28) $eGFR = 144 \times (Q/62)^{-0.329} \times 0.993^{\max(\text{Age}; 40)}$ in females with $Q \leq 62 \mu\text{mol/L}$,
 $eGFR = 144 \times (Q/62)^{-1.209} \times 0.993^{\max(\text{Age}; 40)}$ in females with $Q > 62 \mu\text{mol/L}$,
 $eGFR = 141 \times (Q/80)^{-0.411} \times 0.993^{\max(\text{Age}; 40)}$ in males with $Q \leq 80 \mu\text{mol/L}$ and
 $eGFR = 141 \times (Q/80)^{-1.209} \times 0.993^{\max(\text{Age}; 40)}$ in males with $Q > 80 \mu\text{mol/L}$, where Q represents age-adjusted creatinine (age 2-39) or actual creatinine (age ≥ 40) and $\max(\text{Age}; 40)$ represents the maximum of actual age and 40 years. Q is obtained from $LN(Q) = LN(SCr) + 0.259 \times (40 - \text{Age}) - 0.543 \times \ln^{40}/\text{Age} - 0.00763 \times (40^2 - \text{Age}^2) + 0.0000790 \times (40^3 - \text{Age}^3)$ in males, and $LN(Q) = LN(SCr) + 0.177 \times (40 - \text{Age}) - 0.223 \times \ln^{40}/\text{Age} - 0.00596 \times (40^2 - \text{Age}^2) + 0.0000686 \times (40^3 - \text{Age}^3)$ in females.
- iCARE (29) $eGFR = 50.7 \times BSA^{0.816} \times (L/SCr)^{0.405} \times 0.8994$ if sex = female | 1 otherwise; in which L represents height in cm, SCr in $\mu\text{mol/L}$.

Abbreviations: FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25, LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children; iCARE, Improving renal complications in Adolescents with T2D through Research; SCr, serum creatinine; BSA, body surface area.

Statistical analysis

The basic features of the data in this study were described using descriptive statistics. Summary statistics are presented as mean $\pm$ standard deviation (for normally distributed data) and median (interquartile range) otherwise. Minimum, maximum and percentiles (P5, P25, P75 and P95) are presented for SCr/Q. Distributions of SCr/Q and eGFR-predictions are presented as histograms and quantile regression is used to investigate the age-dependency of SCr/Q and eGFR-predictions. Generalized linear models were used for SCr/Q and all GFR-estimating equations with age (2-year subgroups), sex, IOTF, and their interactions. All statistics were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Clinical Trial registration. ClinicalTrial.gov; Registration Number: NCT02091544.

RESULTS

Characteristics

600 children were included in this study of whom 53.5% was female. Mean age was 12.20 ± 3.28 years. Mean BMI z-score was 3.31 ± 0.75 and the prevalence of overweight, obesity and severe obesity was 21.3%, 44.7% and 34.0% respectively. Mean BSA was 1.78 ± 0.43 m².

Creatinine values for age

In Figure 2.1, SCr is plotted against age for males and females, in which the band represents the reference interval for SCr based on the Hoste polynomials (20). From Figure 2.1 it can be concluded that most of the children have SCr values lying within the reference band. Moreover, there is a slight difference between the bands for males and females, especially for ages > 15 years. In Figure 2.2, SCr/Q ('normalized SCr') is plotted against age with the reference band [0.67 – 1.33] as described by Pottel et al. (21). 96.5% of the children have a SCr/Q within this reference band. There are some children with SCr/Q > 1.33, but they are all still having SCr/Q ≤ 1.5. There is one child with a very low SCr/Q-value (a boy of 17 years and 9 months old with a SCr/Q value of 0.43). Normalizing SCr with the Q-values results in data presented in Table 2.2. Except for the [0-4] year age-group (with only 3 subjects), the median and mean SCr/Q is close to '1' for all age-groups. In general, there is a small but significant difference between males and females (0.97 vs. 1.01) in mean normalized SCr ($p = 0.0006$). As the standard deviation in each subgroup is stable, it can be expected that the SCr/Q values are normally distributed, which is shown in Figure 2.3. The linear quantile regression in the same figure shows a flat median quantile line close to '1'.

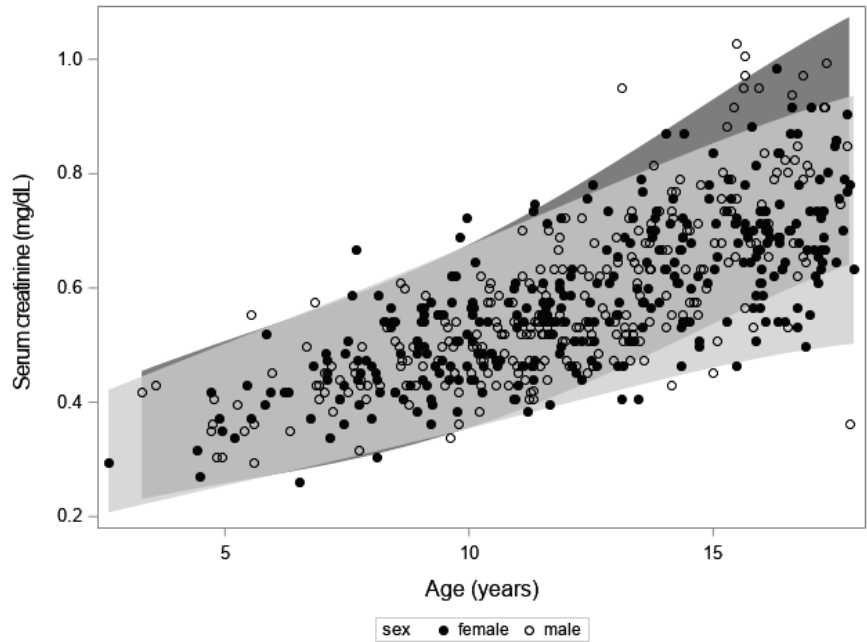


Figure 2.1. Serum creatinine (SCr, in mg/dL) against age for males and females. The band represents the reference interval for SCr based on the Hoste polynomials (20).

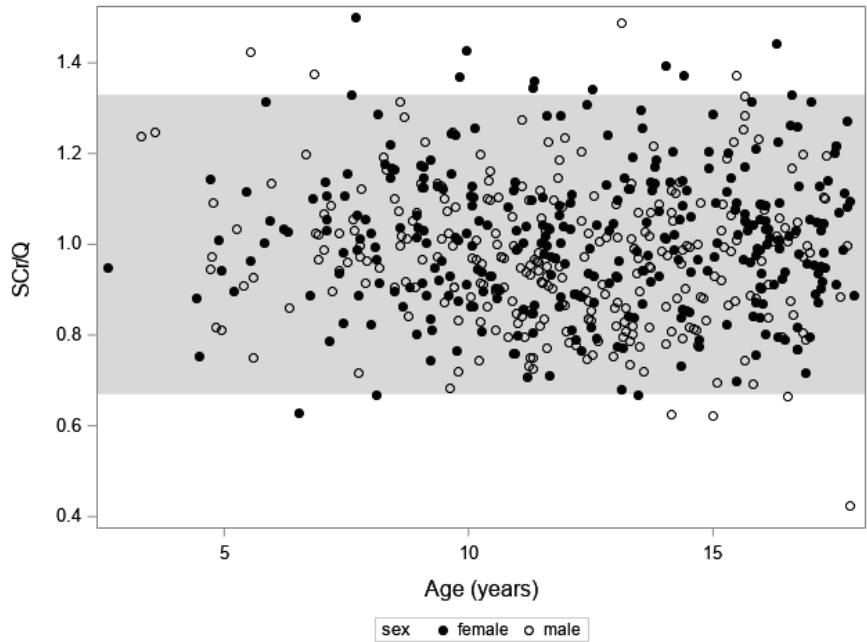


Figure 2.2. SCr/Q against age with the reference band [0.67 – 1.33] as described by Pottel et al. (21). 96.5% of the children have a SCr/Q within this reference band.

Table 2.2. Normalized SCr with the Q-values, stratified per age category.

Age (years)	n	SCr/Q								
		Mean $\pm$ SD	Minimum	P5	P25	Median	P75	P95	Maximum	Quartile range
[0 - 4]	3	1.15 $\pm$ 0.17	0.95	0.95	0.95	1.24	1.25	1.25	1.25	0.30
[4 - 6]	22	1.00 $\pm$ 0.17	0.75	0.76	0.90	0.97	1.09	1.32	1.42	0.20
[6 - 8]	45	1.02 $\pm$ 0.15	0.63	0.79	0.94	1.02	1.07	1.33	1.50	0.12
[8 - 10]	84	1.03 $\pm$ 0.16	0.67	0.77	0.91	1.02	1.14	1.28	1.43	0.23
[10 - 12]	136	0.97 $\pm$ 0.14	0.71	0.75	0.87	0.96	1.06	1.24	1.36	0.18
[12 - 14]	109	0.96 $\pm$ 0.16	0.67	0.75	0.84	0.94	1.07	1.24	1.49	0.23
[14 - 16]	112	0.99 $\pm$ 0.16	0.62	0.73	0.88	0.99	1.09	1.29	1.39	0.22
[16 - 18]	89	1.00 $\pm$ 0.16	0.43	0.77	0.91	1.01	1.09	1.26	1.44	0.17

Abbreviations: SCr/Q = serum creatinine normalized using the Q-age polynomials; n = number of children; P = percentile.

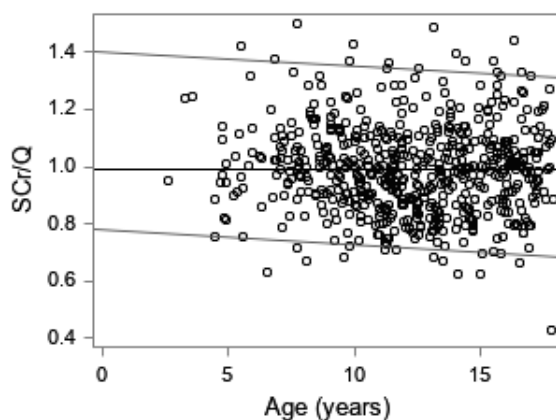


Figure 2.3. Histogram with normal density function overlaid for normalized or rescaled SCr. The vertical reference lines correspond with the lower and upper limits of 0.67 and 1.33 (left). Linear quantile regression lines for percentiles 2.5, 50 and 97.5 are shown (right).

Creatinine-based GFR estimating equations

Knowing the SCr values are within the reference range for age and sex corresponding healthy children, it would be expected that eGFR is age- and sex independent. In Figure 2.4, histograms and linear quantile regression lines for percentiles 2.5, 50 and 97.5 are presented for the creatinine-based GFR estimating equations.

eGFR_{FAS-age} (Figure 2.4A): it is important to note that when SCr/Q is normally distributed, and FAS-age was defined as $107.3/(\text{SCr}/Q)$, thus inversely related to SCr/Q, then it is mathematically impossible that FAS-age is normally distributed. The distribution will be skewed to the right. However, in the literature, it has never been shown that measured GFR shows a distribution skewed to the right. Therefore, this is an artefact of the FAS-equation, and this artefact is especially pronounced when SCr/Q is very low. The outlier

of 0.43 can be seen as the outlier of $107.3/0.43 = 250 \text{ mL/min/1.73m}^2$ (as mentioned earlier). A mean of $\text{SCr}/Q = 1$ is expected ideally, which corresponds with $\text{FAS-age} = 107.3 \text{ mL/min/1.73m}^2$. The vertical lines correspond to $107.3/1.33 = 80.67 \text{ mL/min/1.73m}^2$, that is, the lower limit for FAS-age corresponding to the upper limit of 1.33 for SCr/Q . Symmetrical to the lower limit of $80.67 \text{ mL/min/1.73m}^2$, $134 \text{ mL/min/1.73m}^2$ was defined as the (possible) symmetrical upper limit for FAS-age. However, when $\text{SCr}/Q = 0.67$ (the lower limit for rescaled creatinine) was used, then an upper limit for FAS-age of $160 \text{ mL/min/1.73m}^2$ was found. There is a small but significant difference in eGFR between males and females (113.8 vs $108.6 \text{ mL/min/1.73m}^2$, $p = 0.0006$).

eGFR FAS-height (Figure 2.4B): median values are shifted to higher values, compared to FAS-age, while the extreme outlier is less outlying. This means that higher Q-values are used in the FAS-height equation compared to the FAS-age equation. There is a small but significant difference in eGFR between males and females (118.6 vs $113.4 \text{ mL/min/1.73m}^2$, $p = 0.0021$).

eGFR EKFC (Figure 2.4C): the EKFC-equation is a modified (or optimized) FAS-equation, which solves the flaw of the FAS-equation of large overestimation when SCr/Q -values are low. Median values may not be affected, but the high eGFR predictions by FAS will not be present when EKFC is used. The distribution is more narrow and appears to be skewed to the left (as a consequence of the new power coefficients in the EKFC equation). There is a significant difference in eGFR between males and females (106.8 vs $101.4 \text{ mL/min/1.73m}^2$, $p < 0.0001$).

eGFR bedside Schwartz or CKiD (Figure 2.4D): the histogram is relatively symmetrical, although a little bit skewed to the right. The age-dependent decline is probably an artefact of the Schwartz-equation in children without CKD, due to using a fixed k-value of 0.413 throughout the entire age-range of 2-18 years. This age-dependency of the CKiD (and the Schwartz-Lyon) equation is not unique to our population, as it can also be observed when applying metadata from healthy children in Belgium (1) to these equations (Supplementary Information). This decline with age is even more outspoken in male adolescents. There is no significant difference in eGFR between females and males (112.4 vs $115.2 \text{ mL/min/1.73m}^2$, $p = 0.0858$).

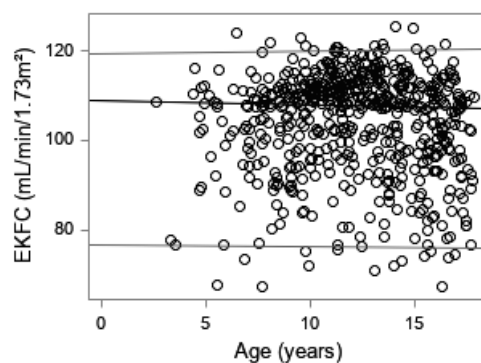
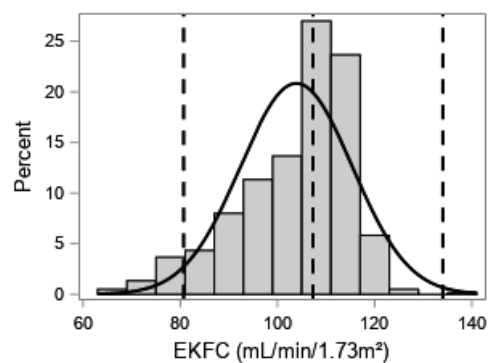
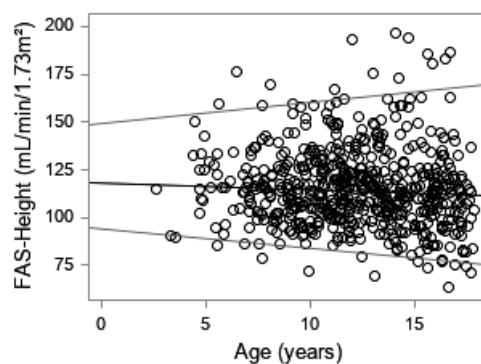
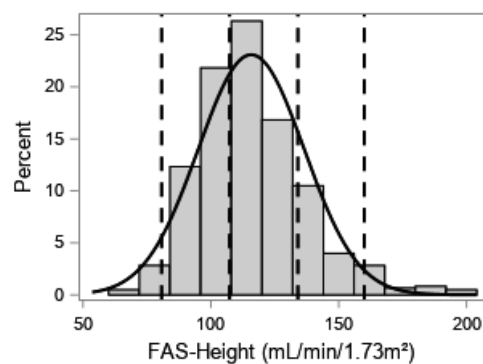
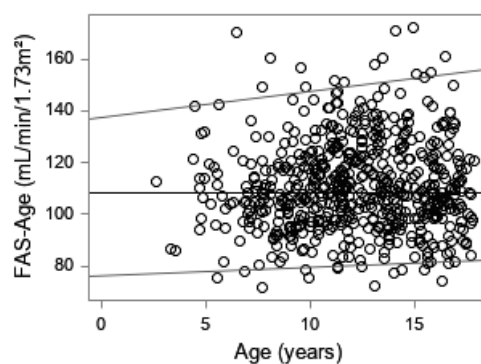
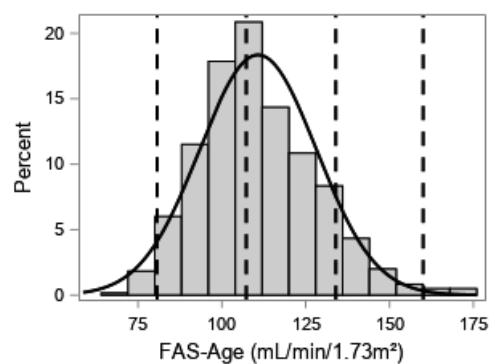
eGFR Schwartz-Lyon (Figure 2.4E): this equation is very similar to the CKiD equation, but mainly differs for females and pre-pubertal males. The same decrease in eGFR is observed as for the eGFR bedside Schwartz equation. Median eGFR-value is about $125 \text{ mL/min/1.73m}^2$ at 2 years of age and decreases to about $90 \text{ mL/min/1.73m}^2$ at 18 years. There is a significant difference in eGFR between males and females (107.2 vs $100.1 \text{ mL/min/1.73m}^2$, $p < 0.0001$).

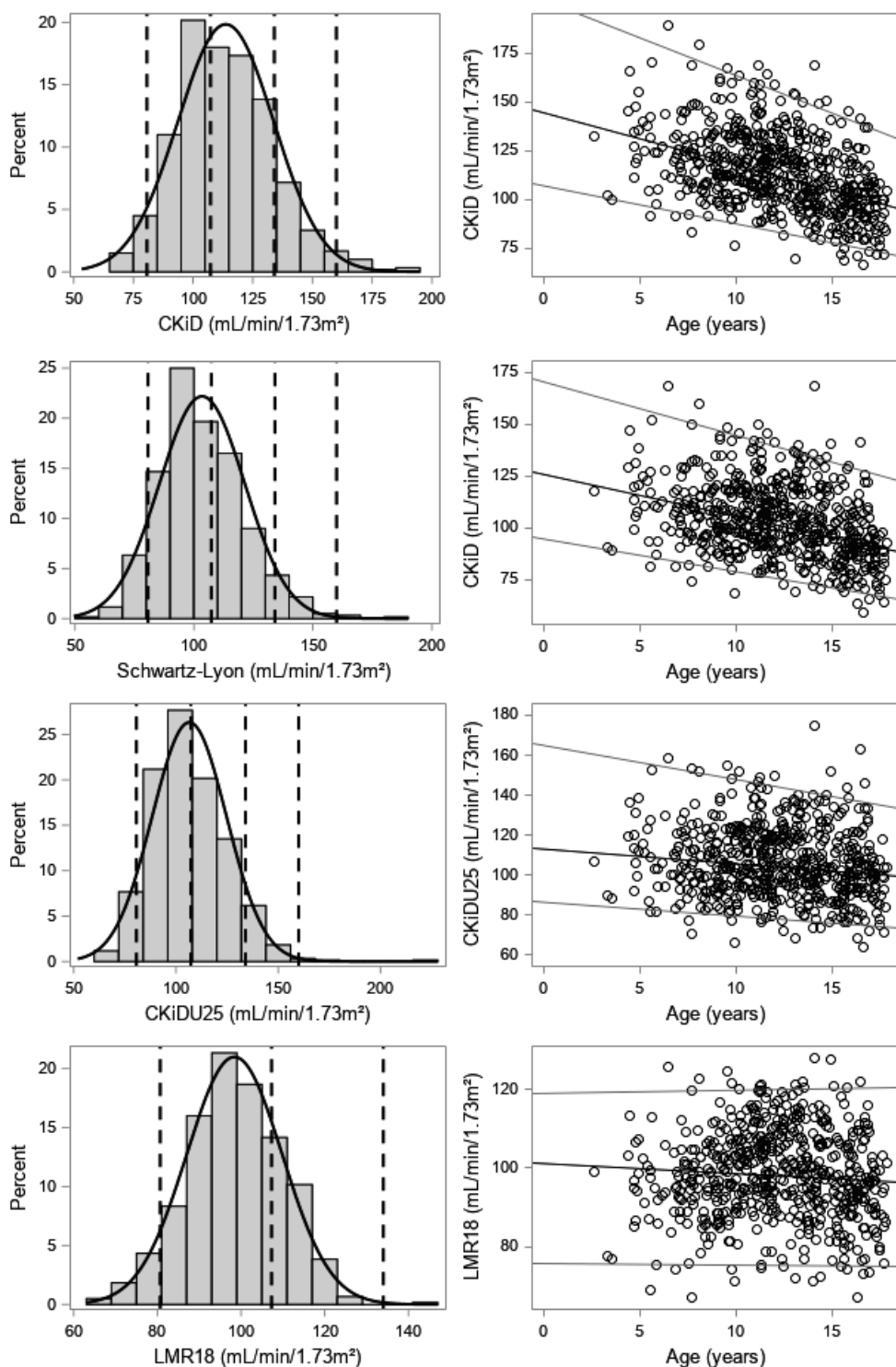
eGFR CKiDU25 (Figure 2.4F): this recently updated CKiD equation no longer showed the decline with age, as a consequence of using age- and sex-dependent k-values. None of the slopes of the quantile lines were significantly different from zero, when studied separately in males and females. In other words, the CKiDU25 equation does not show an age-dependency in our cohort. However, there is a large difference in eGFR between males and females over the whole age spectrum: the mean eGFR is 100.8 mL/min/1.73m² for females and 113.2 mL/min/1.73m² for males ($p < 0.0001$). eGFR values using this CKiDU25 are lower compared to the CKiD equation, especially in children below 10 years of age.

eGFR LMR18 (Figure 2.4G): the histogram is relatively symmetrical and the linear quantile regression lines are flat, indicating that there is no age dependency. Significant difference in eGFR between males and females (101.9 vs 95.4 mL/min/1.73m², $p < 0.0001$).

eGFR CKD-EPI40 for children (Figure 2.4H): this modified CKD-EPI equation does not show an age-dependency, but the distribution of eGFR-values is not symmetrical. Significant difference in eGFR between males and females (104.7 vs 96.6 mL/min/1.73m², $p < 0.0001$).

eGFR iCARE: As a result of the BSA-correction in this equation, eGFR iCARE increases with age which stabilizes after the age of 12 years. In other words: iCARE cannot be used in children of 12 years and younger, but seems applicable for adolescents with overweight and obesity. Remarkable is that iCARE predicts really high eGFR-values for the adolescent group (where it is designed for). No other equation is predicting such high values. Significant difference in eGFR between males and females (127.7 vs 113.4 mL/min/1.73m², $p < 0.0001$).





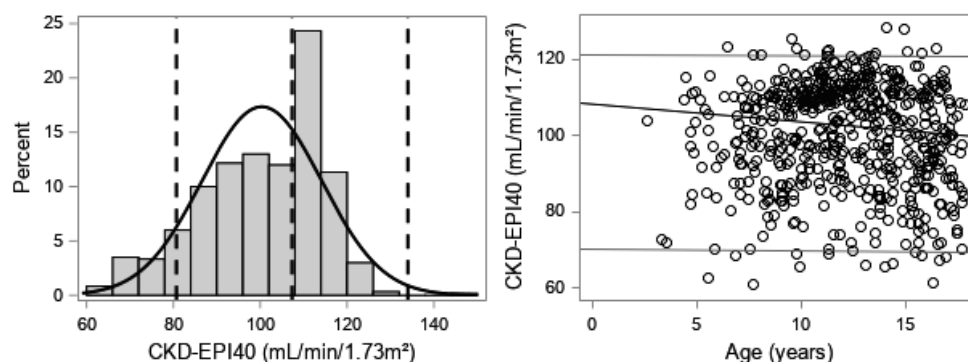


Figure 2.4. Histograms and linear quantile regression of eGFR FAS-age (a), FAS-height (b), EKFC (c), CKiD (bedside Schwartz) (d), Schwartz-Lyon (e), CKiDU25 (f), LMR18 (g), CKD-EPI40 (h). The vertical lines in the histograms correspond to 80.7 (lower limit), 107.3 (median), 133.9 (= symmetrical to 80.7) and 160.1 (upper limit) corresponding to the FAS-eGFR limits calculated from $SCr/Q = 0.67, 1$ and 1.33 as defined by Pottel et al. (21).

Age- and sex-dependency of the creatinine-based GFR-estimating equations

When comparing the young age-group (12 years and younger) with the older age-group (older than 12 years) in females and males, there was no consistency in the results of the different equations. Briefly, we observed a strong age-decline in CKiD and Schwartz-Lyon, but not in the other equations. Most equations showed small differences between sexes, but CKiD and Schwartz-Lyon show no differences between males and females in young children, but substantial differences in adolescents. CKiDU25 shows systematic differences between males and females over the entire age-range. Detailed analyses of the age- and sex-dependency of the creatinine-based GFR-estimating equations can be found in the Supplementary Information.

Creatinine-based GFR-estimating equations and IOTF criteria

In order to examine possible effects of the different IOTF classes (overweight, obesity and severe obesity) on SCr/Q and the different SCr -based GFR-estimating equations, generalized linear models were made for SCr/Q and all GFR-estimating equations (as the dependent variable) with age (2-year subgroups), sex, IOTF, and their interactions (as independent variables). SCr/Q was not affected by age and sex (as supposed), nor by IOTF and their interactions. The height-dependent equations (FAS-height, CKiD, Schwartz-Lyon and CKiDU25) demonstrated a minor but significant IOTF-dependency, with slightly higher eGFR values in children with obesity and severe obesity compared to children with overweight. The age-dependent equations (FAS-age, EKFC, LMR18 and CKD-EPI40) did not show an IOTF-dependency.

DISCUSSION

The main message from this study is that serum creatinine (SCr) values of children with overweight and obesity were mostly within the reference range. Q-age polynomials (the median SCr for healthy children and adolescents) from reference values (20) can be used to normalize serum creatinine ('SCr/Q') in our cohort, resulting in a value that is age- and sex-independent, evidenced by a median and mean SCr/Q close to '1' for all age-groups. As 96.5% of the children with overweight and obesity in this study have a SCr/Q within the reference interval of [0.67 – 1.33] (21), this reference interval can be used to evaluate the normality (or abnormality) of normalized SCr in children with overweight and obesity without overt kidney disease.

Creatinine is the most commonly used endogenous marker for kidney function in children and adults (3). Until approximately two decades ago, reference values for SCr varied between hospitals. Since the implementation of isotope dilution mass spectroscopy (IDMS)-based calibration of creatinine measurement, reference values for SCr in the pediatric population are uniform over the whole age spectrum (19, 30). Normal values for SCr are heavily age-dependent, as SCr increases with muscle mass and (thus) age, with the exception of the neonatal period. Until the age of 14 years, there is no sex-specific difference in SCr, while from the age of 14 years, normal values of SCr in male adolescents are higher than in females (19). Children with overweight and obesity are taller and have an increased absolute fat mass (FM) and fat-free mass (FFM) compared to age- and sex-matched normal weight children; approximately 75% of the excess weight is FM, the remainder FFM (31). Based on this, one should expect higher SCr values in children with overweight and obesity compared to lean children. We here showed that SCr values of children with overweight and obesity are within the reference range. While some studies have demonstrated similar SCr values between children with lean weight and overweight or obesity (32-35), in other studies (36, 37), SCr values were higher in children with overweight or obesity compared to lean controls. It is not excluded that these inconsistent results are due to confounding factors such as age, height and sex and/or due to the heterogeneity of the methods for SCr measurements. Finally, SCr reference values are derived from healthy individuals (19, 30). In both reference studies, anthropometry or body proportion was not mentioned, and thereby the prevalence of overweight or obesity is not known. It is therefore conceivable that SCr reference values are different, possibly lower, in a cohort of exclusively normal-weight children.

Since SCr in our cohort is within the reference range for children, and we did not observe an age and sex-dependency of SCr, it is not expected to see an abnormal kidney function, hence one can expect normal GFR (in ml/min/1.73m^2) and no age/sex-dependency. Also, it is known that measured GFR is stable between 2 and 40 years of age, around 100-110 ml/min/1.73m^2 . Combining these two arguments, it was expected to see a flat

and sex-independent GFR-age relationship for the median and for the quantiles. This is clearly not the case for the CKiD (bedside Schwartz) (24) and Schwartz-Lyon equation (25) which show a decreasing eGFR-age pattern. This age-dependency of the CKiD and Schwartz-Lyon equation is not unique to our population, as it can also be observed when applying metadata from healthy children in Belgium (1) to these equations (Supplementary Information). It can therefore be concluded that these equations, that were developed using data from growth-retarded children with CKD, are not suitable for children of normal height without CKD, with and without overweight and (severe) obesity. The CKiDU25 equation (26) overcomes the age-dependency by using age- and sex-dependent k-values. Though, in our cohort, this equation exhibits a large difference in eGFR between males and females over the whole age spectrum. This sex-difference is deviating from what is observed with other equations and from what is observed in healthy males and females. On the other hand, the iCARE equation (29) shows an increasing eGFR-age pattern. As a result of the BSA-correction in this equation, eGFR iCARE increases with age which stabilizes after the age of 12 years. In other words: iCARE cannot be used in children of 12 years and younger, but seems applicable for adolescents with overweight and obesity. However, it should be mentioned that iCARE predicts very different values for adolescent males and females (a difference of 15 mL/min/1.73m²), and 25-30% higher values compared to the other equations. It should be noted that the iCARE-equation was designed in children with overweight/obesity and type 2 diabetes mellitus, but these large differences require further investigation and the need for measured GFR. FAS-age (22), FAS-height (20) and LMR18 (27) equations show a relatively symmetrical distribution and no age-dependency. The EKFC (23) and CKD-EPI40 formula (28) also do not show an age-dependency, but the distribution of eGFR-values is not symmetrical. There is no consistency between the equations regarding the differences between males and females in young and adolescent age-groups. Finally, the height-dependent equations (FAS-height, CKiD, Schwartz-Lyon and CKiDU25) indicate a minor but statistically significant higher eGFR in children with obesity and severe obesity, compared to children with overweight. The age-dependent equations (FAS-age, EKFC, LMR18 and CKD-EPI) do not show such a dependency. Considering all of this, we suggest that eGFR FAS-age, FAS-height and LMR18 can be allotted as the preferred eGFR equations in children with overweight and obesity.

This study is not without limitations. First and most important, it lacks a gold standard measurement of GFR. It is therefore impossible to state whether the preferred creatinine-based GFR-estimating equation can reflect real kidney function in children with overweight and obesity. In adults with overweight and obesity, the random error of creatinine-based GFR-estimating equation is wide; 90% of the estimations had an error within $\pm 55\%$ of measured GFR, determined using plasma iothexol disappearance (2). Another limitation of this study is the lack of another endogenous marker for kidney function, such as cystatin C. Where creatinine heavily depends on muscle mass, this does

not apply to cystatin C (38). Though, cystatin C synthesis is upregulated in adipose tissue in case of obesity (39) and cystatin C does not improve reliability of GFR estimation over SCr in adults with overweight and obesity (2). However, there are studies in children in which cystatin C improves the predictive performance of eGFR equations (3). Finally, the approach in which SCr- and a cystatin C-based eGFR are compared, instead of combined into more complex equations, can add confidence to the accuracy of the GFR (40) and can have some other advantages as well (3).

So, we suggest that future research implements other endogenous markers for kidney function, such as cystatin C, and measured GFR in order to examine the role of GFR-estimating equations in children with overweight and obesity. Possibly, such studies could as well contribute to our understanding whether GFR-indexation with BSA, or de-indexation of eGFR is helpful in children with overweight and obesity; a topic that is controversial in individuals with altered body proportions (12, 41).

In conclusion, SCr values of children with overweight and obesity without overt kidney disease are mostly within the reference range. Normalization of SCr using reference Q-age polynomials works very well in this cohort. After evaluation of the different equations, we suggest that FAS-age, FAS-height and LMR18 are the preferred creatinine-based GFR-estimating equations in children with overweight and obesity.

REFERENCES

1. Pottel H (2017) Measuring and estimating glomerular filtration rate in children. *Pediatr Nephrol* 32:249-263
2. López-Martínez M, Luis-Lima S, Morales E, Navarro-Díaz M, Negrín-Mena N, Folgueras T, Escamilla B, Estupiñán S, Delgado-Mallén P, Marrero-Miranda D, González-Rinne A, Miquel-Rodríguez RM, Cobo-Caso MA, Díaz-Martín L, Jiménez-Sosa A, González-Rinne F, Torres A, Porrini E (2020) The estimation of GFR and the adjustment for BSA in overweight and obesity: a dreadful combination of two errors. *Int J Obes (Lond)* 44:1129-1140
3. den Bakker E, Gemke R, Bokenkamp A (2018) Endogenous markers for kidney function in children: a review. *Crit Rev Clin Lab Sci* 55:163-183
4. Ebert N, Bevc S, Bökenkamp A, Gaillard F, Hornum M, Jager KJ, Mariat C, Eriksen BO, Palsson R, Rule AD, van Londen M, White C, Schaeffner E (2021) Assessment of kidney function: clinical indications for measured GFR. *Clin Kidney J* 14:1861-1870
5. Wyss M, Kaddurah-Daouk R (2000) Creatine and creatinine metabolism. *Physiol Rev* 80:1107-1213
6. Schloerb PR (1960) Total body water distribution of creatinine and urea in nephrectomized dogs. *Am J Physiol* 199:661-665
7. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, Heilberg IP (2008) Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol.* 3:348-354
8. Schwartz GJ, Work DF (2009) Measurement and estimation of GFR in children and adolescents. *Clin J Am Soc Nephrol.* 4:1832-1843
9. Piepsz A, Tondeur M, Ham H (2006) Revisiting normal (51)Cr-ethylenediaminetetraacetic acid clearance values in children. *Eur J Nucl Med Mol Imaging* 33:1477-1482
10. Piepsz A, Tondeur M, Ham H (2008) Escaping the correction for body surface area when calculating glomerular filtration rate in children. *Eur J Nucl Med Mol Imaging* 35:1669-1672
11. Pottel H, Mottaghy FM, Zaman Z, Martens F (2010) On the relationship between glomerular filtration rate and serum creatinine in children. *Pediatr Nephrol* 25:927-934
12. Delanaye P, Radermecker RP, Rorive M, Depas G, Krzesinski JM (2005) Indexing glomerular filtration rate for body surface area in obese patients is misleading: concept and example. *Nephrol Dial Transplant* 20:2024-2028
13. Chang AR, Zafar W, Grams ME (2018) Kidney Function in Obesity-Challenges in Indexing and Estimation. *Adv Chronic Kidney Dis* 25:31-40
14. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, Praga M (2016) Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol* 12:453-471
15. Jadresic L, Silverwood RJ, Kinra S, Nitsch D (2019) Can childhood obesity influence later chronic kidney disease? *Pediatr Nephrol* 34:2457-2477
16. van Dam M, Rijks J, Dorenbos E, Horuz F, van Dael K, Vreugdenhil A (2019) The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity. *Sci Rep.* 9:4504
17. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 7:284-294
18. Haycock GB, Schwartz GJ, Wisotsky DH (1978) Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr* 93:62-66

19. Pottel H, Vrydags N, Mahieu B, Vandewynckele E, Croes K, Martens F (2008) Establishing age/sex related serum creatinine reference intervals from hospital laboratory data based on different statistical methods. *Clin Chim Acta* 396:49-55
20. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aïssa A, Cochat P, Martens F, Pottel H (2014) A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant* 29:1082-1091
21. Pottel H, Hoste L, Delanaye P (2015) Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m². *Pediatr Nephrol* 30:821-828
22. Pottel H, Hoste L, Martens F (2012) A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol* 27:973-979
23. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, Dalton RN, Dubourg L, Gaillard F, Garrouste C, Grubb A, Jacquemont L, Hansson M, Kamar N, Lamb EJ, Legendre C, Littmann K, Mariat C, Melsom T, Rostaing L, Rule AD, Schaeffner E, Sundin PO, Turner S, Bökenkamp A, Berg U, Åsling-Monemi K, Selistre L, Åkesson A, Larsson A, Nyman U, Delanaye P (2021) Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate : A Cross-sectional Analysis of Pooled Data. *Ann Intern Med* 174:183-191
24. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL (2009) New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 20:629-637
25. Selistre L, De Souza V, Cochat P, Antonello IC, Hadj-Aïssa A, Ranchin B, Dolomanova O, Varennes A, Beyerle F, Bacchetta J, Dubourg L (2012) GFR estimation in adolescents and young adults. *J Am Soc Nephrol* 23:989-996
26. Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ (2021) Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. *Kidney Int.* 99:948-956
27. Björk J, Nyman U, Delanaye P, Grubb A, Larsson A, Vranken L, Åkesson A, Pottel H (2020) A novel method for creatinine adjustment makes the revised Lund-Malmö GFR estimating equation applicable in children. *Scand J Clin Lab Invest* 80:456-463
28. Björk J, Nyman U, Larsson A, Delanaye P, Pottel H (2021) Estimation of the glomerular filtration rate in children and young adults by means of the CKD-EPI equation with age-adjusted creatinine values. *Kidney Int.* 99:940-947
29. Dart AB, McGavock J, Sharma A, Chateau D, Schwartz GJ, Blydt-Hansen T (2019) Estimating glomerular filtration rate in youth with obesity and type 2 diabetes: the iCARE study equation. *Pediatr Nephrol* 34:1565-1574
30. Ceriotti F, Boyd JC, Klein G, Henny J, Queraltó J, Kairisto V, Panteghini M (2008) Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem* 54:559-566
31. Wells JC, Fewtrell MS, Williams JE, Haroun D, Lawson MS, Cole TJ (2006) Body composition in normal weight, overweight and obese children: matched case-control analyses of total and regional tissue masses, and body composition trends in relation to relative weight. *Int J Obes (Lond)* 30:1506-1513
32. Bostan Gayret Ö, Taşdemir M, Erol M, Tekin Nacaroglu H, Zengi O, Yiğit Ö (2018) Are there any new reliable markers to detect renal injury in obese children? *Renal failure* 40:416-422
33. Önerli Salman D, Şıklar Z, Çullas İlarıslan EN, Özçakar ZB, Kocaay P, Berberoğlu M (2019) Evaluation of Renal Function in Obese Children and Adolescents Using Serum Cystatin C Levels, Estimated Glomerular Filtration Rate Formulae and Proteinuria: Which is most Useful? *J Clin Res Pediatr Endocrinol.* 11:46-54

34. Martin-Del-Campo F, Batis-Ruvalcaba C, Ordaz-Medina SM, Martínez-Ramírez HR, Vizmanos-Lamotte B, Romero-Velarde E, Cortes-Sanabria L, Cueto-Manzano AM (2019) Frequency and Risk Factors of Kidney Alterations in Children and Adolescents who Are Overweight and Obese in a Primary Health-care Setting. *J Ren Nutr* 29:370-376
35. Murphy MO, Huang H, Bauer JA, Schadler A, Makhoul M, Clasey JL, Chishti AS, Kiessling SG (2021) Impact of Pediatric Obesity on Diurnal Blood Pressure Assessment and Cardiovascular Risk Markers. *Front Pediatr* 9:596142
36. Correia-Costa L, Schaefer F, Afonso AC, Bustorff M, Guimarães JT, Guerra A, Barros H, Azevedo A (2016) Normalization of glomerular filtration rate in obese children. *Pediatr Nephrol* 31:1321-1328
37. Morato M, Correia-Costa L, Sousa T, Cosme D, Schaefer F, Areias JC, Guerra A, Afonso AC, Barros H, Azevedo A, Albino-Teixeira A (2017) Longer duration of obesity is associated with a reduction in urinary angiotensinogen in prepubertal children. *Pediatr Nephrol* 32:1411-1422
38. Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Brodehl J (1998) Reference values for cystatin C serum concentrations in children. *Pediatr Nephrol* 12:125-129
39. Dedual MA, Wueest S, Challa TD, Lucchini FC, Aeppli TRJ, Borsigova M, Mauracher AA, Vavassori S, Pachlopnik Schmid J, Blüher M, Konrad D (2020) Obesity-Induced Increase in Cystatin C Alleviates Tissue Inflammation. *Diabetes* 69:1927-1935
40. den Bakker E, Gemke R, van Wijk JAE, Hubeek I, Stoffel-Wagner B, Bökenkamp A (2018) Combining GFR estimates from cystatin C and creatinine-what is the optimal mix? *Pediatr Nephrol* 33:1553-1563
41. Delanaye P, Krzesinski JM (2011) Indexing of renal function parameters by body surface area: intelligence or folly? *Nephron Clin Pract* 119:c289-292.

SUPPLEMENTARY INFORMATION

Age-dependency of the creatinine-based GFR-estimating equations

In order to evaluate the age-dependency in more detail, we used two age groups: a) ≤ 12 years and b) > 12 years and calculated descriptive statistics for both age-groups, displayed in Table S2.1. For FAS-age, FAS-height, EKFC, CKiDU25, LMR18 and CKD-EPI40, the difference between the mean (or median) values is small, mostly less than 2-3 mL/min/1.73m². However, this is clearly different for the CKiD (bedside Schwartz), Schwartz-Lyon and iCARE equations where large differences (10-15 mL/min/1.73m² for CKiD and close to 30 mL/min/1.73m² for iCARE) between the means (or medians) of the age-groups are observed, with a lower mean value for the Schwartz equations in the > 12 -year age group, but contrary, with a higher mean value for iCARE, compared to the ≤ 12 -year age group. For iCARE, it can be explained as this equation should not be used in the ≤ 12 -year age group.

Supplementary Information Table S2.1. Descriptive statistics of the different creatinine-based GFR-estimating equations stratified according to age group ≤ 12 years and > 12 years.

Age group	n	Equations	Mean $\pm$ SD	P5	P25	P50	P75	P95	Quartile range
≤ 12 years	290	FAS-age	109.9 $\pm$ 16.6	84.1	97.7	108.4	118.7	142.0	21.0
		FAS-height	116.3 $\pm$ 18.2	90.2	103.3	115.2	126.7	151.3	23.4
		EKFC	104.5 $\pm$ 10.8	81.3	98.8	108.1	111.6	117.3	12.8
		CKiD	121.2 $\pm$ 18.6	94.0	107.7	119.6	132.6	155.2	24.9
		Schwartz-Lyon	108.0 $\pm$ 16.6	83.8	96.0	106.6	118.2	138.3	22.2
		CKiDU25	107.9 $\pm$ 17.0	82.5	94.9	105.9	119.3	138.5	24.4
		LMR18	98.8 $\pm$ 10.7	80.6	92.2	99.0	105.9	116.0	13.8
		CKD-EPI40	101.1 $\pm$ 13.0	76.3	92.7	104.3	110.7	117.8	18.0
		iCARE	105.5 $\pm$ 19.7	75.9	91.6	104.6	119.5	139.4	28.0

Supplementary Information Table S2.1. Descriptive statistics of the different creatinine-based GFR-estimating equations stratified according to age group ≤ 12 years and > 12 years. (*continued*)

Age group	n	Equations	Mean $\pm$ SD	P5	P25	P50	P75	P95	Quartile range
> 12 years	310	FAS-age	112.1 $\pm$ 19.8	84.8	98.6	108.6	123.3	142.4	24.7
		FAS-height	115.3 $\pm$ 22.8	83.3	100.9	113.4	124.4	158.3	23.6
		EKFC	103.3 $\pm$ 12.1	78.6	96.0	107.3	112.1	117.7	16.1
		CKiD	106.7 $\pm$ 18.9	79.3	93.6	104.0	118.5	140.0	24.9
		Schwartz-Lyon	99.1 $\pm$ 18.2	72.6	86.4	96.4	109.6	131.0	23.1
		CKiDU25	105.3 $\pm$ 19.2	77.8	93.0	102.9	117.7	136.2	24.8
		LMR18	98.0 $\pm$ 12.0	77.0	90.0	97.3	107.0	116.5	17.1
		CKD-EPI40	99.6 $\pm$ 14.5	72.0	89.2	101.4	111.3	118.2	22.1
		iCARE	133.7 $\pm$ 19.5	104.6	121.3	132.0	144.5	165.0	23.2

Note that iCARE should not be used in children ≤ 12 years of age (as it was designed for adolescents). Abbreviations: n = number of children; P = percentile; FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25, LMR18, revised Lund-Malmö; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; iCARE, Improving renal complications in Adolescents with T2D through Research; SCr, serum creatinine.

Age-dependency of the CKiD and iCARE equations using metadata

In order to examine whether the age-dependency of the CKiD and iCARE is unique to our population, metadata from healthy children in Belgium (1) were applied to these equations. It is clear that eGFR CKiD decreases with age, which is not expected in healthy children, nor in children with overweight and obesity (since SCr are comparable with normal reference values). This age-dependency is more outspoken in male adolescents, and is the result of the age- and sex-independent fixed k-value of 0.413. The eGFR CKiDU25, that uses an age and sex-dependent k-value, does not show this age-dependency anymore. Though, the CKiDU25 shows a remarkable sex difference. Since this topic is beyond the scope of this article, the interested reader is referred to an upcoming article.

In Figure S2.1, eGFR-iCARE is plotted against age. It is clear that eGFR iCARE increases with age which stabilizes in adolescents. In other words: iCARE cannot be used in the full-age spectrum in children, but seems applicable for adolescents.

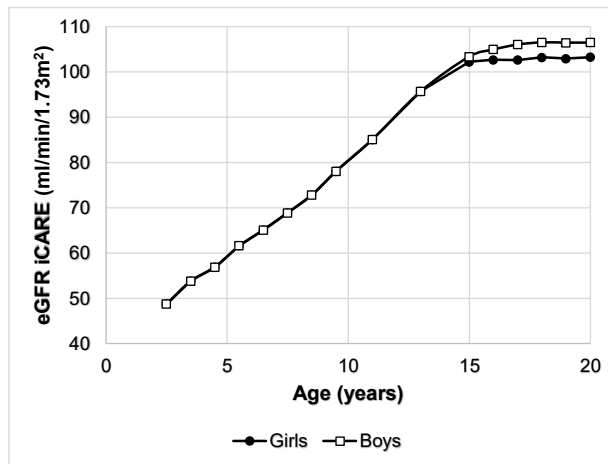


Figure S2.1. eGFR-iCARE (ml/min/1.73m²) plotted against age (years) when applying metadata from healthy children in Belgium (1) to this equation.

Sex differences between the creatinine-based GFR-estimating equations

We analysed sex differences using the same age groups, as displayed in Table S2.2 (for children ≤ 12 years) and Table S2.3 (for children > 12 years). SCr/Q is equal to 1.01 (female, ≤ 12 years), 0.99 (female, > 12 years), 1.01 (male ≤ 12 years), and 0.95 (male, > 12 years) respectively. As these differences are small, this is reflected in small differences of mean FAS-age: 108.9, 110.9, 108.4 and 116.8 mL/min/1.73m², and in FAS-height: 115.3, 117.3, 111.7 and 119.9 mL/min/1.73m². Slightly higher eGFR in males ≤ 12 years than in females ≤ 12 years, and mildly higher in male adolescents. Note that the Q-values for FAS do not differ between males and females in the young age-group (1), therefore, it is not expected to see large differences between females and males in this age-group. LMR18 and CKD-EPI40 show higher values (around 5-7 mL/min/1.73m²) in males than in females, but no real difference between the young and adolescent age-groups. The CKiD (bedside Schwartz) equation shows a totally different picture: no real difference between males and females within the same age-group, but higher in the young age group (around 120 mL/min/1.73m²) than in the adolescent age-group (around 105-108 mL/min/1.73m²). Schwartz-Lyon shows no difference between males and females in the young age-group but a large difference in the adolescent age group (around 11 mL/min/1.73m²). Note that Schwartz-Lyon uses different k-values in adolescent females and males, which may explain the difference. CKiDU25 shows large differences between males and females (around 11 mL/min/1.73m²) in both age-groups, but much smaller differences between sex-specific age-groups. iCARE (if only considering the > 12 -year age-group) shows a clear difference between females and males, 126.5 and 143 mL/min/1.73m² respectively. Compared to the other equations, iCARE shows significantly higher eGFR-predictions, probably the effect of the BSA-correction in this equation.

Supplementary Table S2.2. Descriptive statistics of the different creatinine-based GFR-estimating equations stratified according to sex for males and females of 12 years and younger.

Sex	n	Variables	Mean $\pm$ SD	P5	P25	P50	P75	P95	Quartile range
Female	146	SCr/Q	1.01 $\pm$ 0.16	0.76	0.90	1.00	1.11	1.29	0.21
		FAS-age	108.9 $\pm$ 17.1	83.4	96.8	106.8	119.5	141.0	22.7
		FAS-height	115.3 $\pm$ 19.5	89.6	101.9	111.6	126.7	151.3	24.8
		EKFC	102.8 $\pm$ 11.3	80.4	94.6	106.3	110.9	117.0	16.3
		CKiD	120.5 $\pm$ 19.9	91.7	106.7	117.0	133.9	159.6	27.2
		Schwartz-Lyon	107.3 $\pm$ 17.8	81.7	95.0	104.3	119.3	142.2	24.4
		CKiDU25	103.0 $\pm$ 16.9	79.7	91.5	100.3	113.1	136.2	21.6
		LMR18	96.6 $\pm$ 10.7	78.5	89.0	96.3	103.9	114.8	15.0
		CKD-EPI40	98.3 $\pm$ 13.4	73.7	87.7	99.3	110.0	116.2	22.3
		iCARE	97.7 $\pm$ 17.5	72.9	84.4	96.5	108.1	128.7	23.7
Male	144	SCr/Q	0.99 $\pm$ 0.14	0.75	0.91	0.97	1.08	1.24	0.18
		FAS-age	110.9 $\pm$ 16.0	86.6	98.9	110.5	118.4	142.8	19.5
		FAS-height	117.3 $\pm$ 16.9	91.7	105.5	116.4	126.7	150.3	21.2
		EKFC	106.2 $\pm$ 10.1	86.2	101.8	109.3	112.2	119.6	10.4
		CKiD	122.0 $\pm$ 17.2	96.0	109.5	121.7	132.3	153.5	22.9
		Schwartz-Lyon	108.7 $\pm$ 15.3	85.6	97.5	108.4	117.9	136.8	20.4
		CKiDU25	112.9 $\pm$ 15.7	89.4	101.5	112.5	122.0	143.6	20.5
		LMR18	101.1 $\pm$ 10.3	84.3	94.7	101.8	107.3	119.5	12.6
		CKD-EPI40	104.0 $\pm$ 11.9	81.4	97.3	107.7	111.5	120.8	14.2
		iCARE	113.4 $\pm$ 18.7	81.5	101.4	114.9	124.0	145.1	22.6

Note that iCARE was not designed for children ≤ 12 years of age. Abbreviations: SCr/Q = serum creatinine normalized using the Q-age polynomials; n = number of children; P = percentile; FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25, LMR18, revised Lund-Malmö; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; iCARE, Improving renal complications in Adolescents with T2D through Research; SCr, serum creatinine.

Supplementary Table S2.3. Descriptive statistics of the different creatinine-based GFR-estimating equations stratified according to sex for males and females older than 12 years.

Sex	n	Variables	Mean $\pm$ SD	P5	P25	P50	P75	P95	Quartile range
Female	175	SCr/Q	1.01 $\pm$ 0.15	0.77	0.91	1.01	1.11	1.30	0.20
		FAS-age	108.4 $\pm$ 16.9	82.8	96.5	105.8	117.6	138.9	21.1
		FAS-height	111.7 $\pm$ 22.0	82.1	96.4	110.5	120.1	152.9	23.7
		EKFC	100.2 $\pm$ 11.9	78.0	92.4	102.2	109.5	115.9	17.1
		CKiD	105.6 $\pm$ 18.2	80.4	93.4	103.5	116.3	142.0	22.8
		Schwartz-Lyon	94.1 $\pm$ 16.2	71.6	83.2	92.2	103.6	126.5	20.3
		CKiDU25	98.9 $\pm$ 15.8	76.6	87.5	97.7	107.3	132.7	19.8
		LMR18	94.3 $\pm$ 10.9	76.5	87.5	93.9	101.2	113.0	13.8
		CKD-EPI40	95.2 $\pm$ 14.0	71.3	85.5	95.3	108.2	115.1	22.7
		iCARE	126.5 $\pm$ 16.0	101.5	115.2	125.5	138.0	150.7	22.8
Male	135	SCr/Q	0.95 $\pm$ 0.16	0.72	0.82	0.95	1.05	1.23	0.23
		FAS-age	116.8 $\pm$ 22.1	87.5	102.1	112.9	130.6	149.0	28.5
		FAS-height	120.0 $\pm$ 23.2	86.3	105.9	116.5	129.5	162.9	23.6
		EKFC	107.4 $\pm$ 11.0	83.8	102.1	110.0	114.9	120.2	12.8
		CKiD	108.0 $\pm$ 19.8	78.8	94.6	106.0	120.3	140.0	25.7
		Schwartz-Lyon	105.7 $\pm$ 18.7	78.8	93.2	104.0	118.5	135.7	25.3
		CKiDU25	113.6 $\pm$ 20.0	85.8	100.8	111.4	124.2	143.5	23.4
		LMR18	102.8 $\pm$ 11.8	82.5	94.8	102.7	111.9	120.5	17.1
		CKD-EPI40	105.4 $\pm$ 13.1	79.0	97.5	108.3	114.8	121.6	17.3
		iCARE	143.0 $\pm$ 19.8	116.1	129.0	140.2	152.6	182.9	23.6

Abbreviations: SCr/Q = serum creatinine normalized using the Q-age polynomials; n = number of children; P = percentile; FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25; LMR18, revised Lund-Malmö; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; iCARE, Improving renal complications in Adolescents with T2D through Research; SCr, serum creatinine.

SUPPLEMENTARY REFERENCE

1. Pottel H (2017) Measuring and estimating glomerular filtration rate in children. *Pediatr Nephrol* 32:249-263

3

Relation between obesity-related comorbidities and kidney function estimation in children

Mark J.C.M. van Dam, Hans Pottel, Anita C.E. Vreugdenhil

Pediatr Nephrol. 2023 Jun;38(6):1867-1876

ABSTRACT

Background. The current childhood obesity pandemic is likely to result in an increased risk of chronic kidney disease (CKD) later in life. Correlations between obesity-related comorbidities and kidney function can be found, but it is unclear to what extent this is caused by bias due to different mathematical forms of the estimated glomerular filtration rate (eGFR) equations. The present study aimed to analyze correlations between obesity-related comorbidities and different eGFR-equations, and to investigate whether rescaled serum creatinine (SCr/Q) for sex and age or height might be an alternative biomarker for kidney function estimation.

Methods. This cross-sectional cohort study included 600 children with overweight and obesity. Mean age was 12.2 ± 3.28 years, 53.5% was female, and mean BMI z-score was 3.31 ± 0.75 . All children underwent a comprehensive assessment that included anthropometrical and blood-pressure measurements, laboratory examination, air displacement plethysmography, and polysomnography. Q-age and Q-height polynomials were used to rescale SCr and multiple creatinine-based eGFR-equations were compared.

Results. SCr/Q and almost all GFR estimations significantly correlated with waist to hip ratio, fat mass, homeostasis model assessment for insulin resistance, triacylglyceride, HDL cholesterol, alanine transaminase and serum uric acid concentrations. Multiple correlations however were not confirmed by all equations, which suggests dependency on the mathematical form of the different eGFR-equations.

Conclusions. Correlations between obesity-related comorbidities and creatinine-based eGFR are present in children with overweight and obesity, but depend to a large extent on the eGFR-equation of choice. SCr/Q might be an alternative biomarker for assessing correlations between obesity-related comorbidities and kidney function in children with overweight and obesity.

INTRODUCTION

The global prevalence of children with overweight and obesity has dramatically increased during the last few decades (1). Obesity during childhood increases the risk of developing various comorbidities, including diabetes mellitus, hypertension and dyslipidemia (2). Additionally, children with obesity are prone to become adults with obesity, increasing the lifetime health risk even more (3). With the first report that dates from 1974 describing an association between severe obesity and nephrotic-range proteinuria (4), nowadays the relation between obesity and chronic kidney disease (CKD) is widely accepted (5-7). Apart from the separate entity called obesity-related glomerulopathy, pathologically defined as glomerular hypertrophy and adaptive focal segmental glomerulosclerosis, obesity is an important prognostic factor for an adverse kidney outcome in patients with a solitary functioning kidney, IgA nephropathy and autosomal dominant polycystic disease for example (8-10). So, obesity therefore plays a major role in the increasing prevalence of CKD, a condition that already 10% of the population worldwide suffers from (11).

The current pandemic of childhood obesity is likely to result in an increased risk of CKD in later life, as early-life adiposity is associated with CKD on the long term (12-14). Unfortunately, kidney injury due to obesity might go unnoticed for years, hence named a 'silent' comorbidity of obesity, with a consequence of delayed diagnosis and therefore impaired kidney prognosis.

Although the pathophysiology of obesity-related kidney injury is not fully understood, there seems to be a major role of glomerular hyperfiltration, increased activity of the renin-angiotensin-aldosterone system, insulin resistance and lipid accumulation in the kidney (6, 7). Since these alterations in the kidney occur before kidney dysfunction or the detection of microalbuminuria or hypertension (15), these markers might not be useful in the early phase of obesity-related kidney injury, and so there is need for other biomarkers. In children with obesity, urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), both markers for proximal tubular cell injury, might be used for the early diagnosis of kidney injury (16). In adolescents with obesity with a cystatin-C based glomerular filtration rate (GFR) ≤ 130 ml/min/1.73m² increased urinary NGAL concentrations might predict CKD (17). Since these biomarkers are not (yet) implemented in clinical practice, most clinical studies on childhood obesity and kidney function use creatinine-based estimated glomerular filtration rate (eGFR) and (micro)albuminuria as markers for kidney health (13). However, eGFR is almost always preserved in children with overweight and obesity without a history of kidney disease (18, 19), and microalbuminuria has a prevalence that ranges from 0.3 to 7.9%, which in fact is not different from the prevalence in lean children (20). Clinical studies examining associations between obesity-related comorbidities and kidney function in childhood have yielded inconsistent results (13). Important to consider is that eGFR-equations using

endogenous filtration markers are suffering from inaccuracy and imprecision (21) and not all creatinine-based GFR estimating equations seem suitable in children with overweight and obesity (19). Considering all of this, GFR-estimating equations might be inappropriate for evaluating correlations between kidney function and 'metabolic health' in children with overweight and obesity. We postulate that the discrepant results in other studies examining associations between childhood obesity and kidney disease might be partially caused by bias and random error in eGFR-equations. Therefore, in this study in children with overweight and obesity without overt kidney disease, we will compare different creatinine-based GFR estimating equations and their correlations with anthropometric variables and obesity-related comorbidities. Additionally, we will examine whether rescaled SCr for sex and age or height might be used as an alternative marker.

METHODS

Study population

This study uses baseline, pre-intervention data from the Centre of Overweight Adolescent and Children's Healthcare (COACH): a clinical multidisciplinary lifestyle intervention program at the Maastricht University Medical Centre + (MUMC+). In this program, children with overweight, obesity and severe obesity are evaluated to identify the underlying cause of the adiposity and potential obesity-related comorbidities, as described previously (19, 22). Between January 1st 2011 and April 1st 2019 662 children entered the COACH program. Exclusion criteria for this study were age $\geq$ 18 years ($n=5$), normal weight (at the moment of the measurements in our centre ($n=5$)), secondary cause of adiposity, known kidney disease (congenital or acquired), diabetes mellitus and/or use of antihypertensive medication ($n=13$). Serum creatinine (SCr) was not measured in 39 children. All other children ($n=600$) were included in this study. The Medical Ethical Committee of the MUMC+ approved this study and informed consent was obtained.

Clinical assessment, anthropometry and body composition

After entering the COACH program, children were admitted for approximately 24 hours at the pediatric department of the MUMC+ for clinical evaluation. While children wore only underwear, weight and height were determined using a digital scale (Seca, Chino, CA) and stadiometer (De Grood Metaaltechniek, Nijmegen, the Netherlands) respectively. Weight and height were used to calculate body mass index (BMI) and BMI was converted into z-scores. Definition of the International Obesity Task Force (IOTF) were used to define overweight, obesity and severe obesity (23). Waist- and hip-circumference were determined, waist-to-hip ratio was calculated, and waist- and hip-circumference z-scores were obtained using reference values (24). The equation described by Haycock et al. was used to calculate body surface area (BSA) (25). In a subgroup of randomly chosen children

(n=285), fat mass and fat-free mass was determined by air displacement plethysmography (BodPod).

Creatinine-based eGFR equations and microalbuminuria

Serum creatinine (SCr) was measured enzymatically. (Cobas 8000, Roche) Since SCr is heavily dependent on sex, age and height (26, 27), SCr was rescaled using Q-age and Q-height polynomials obtained from the literature (28, 29). SCr/Q ('rescaled SCr') is sex- and age- or height-independent for healthy subjects, depending on whether Q-age or Q-height is used. In a previous study, it was concluded that this concept of rescaling SCr works very well in children with overweight and obesity (19). Rescaled SCr was evaluated using the reference band of [0.67 – 1.33], which represents the 2.5th and 97.5th percentile (30). The following SCr-based eGFR equations were evaluated, as described previously (19): FAS-age (28); FAS-height (29); EKFC (31); updated bedside Schwartz (32); Schwartz-Lyon (33); CKiDU25 (34); LMR18 (35) and CKD-EPI40 (36). Urinary albumin was determined in a random urine spot and the urine albumin-to-creatinine ratio (UACR) was calculated. Microalbuminuria was present when the UACR was between 30 and 300 mg/mg.

Laboratory analyses

Venous blood samples were collected while children were in a fasting state. Fasting plasma glucose, serum uric acid, total cholesterol, high-density lipoprotein (HDL) cholesterol, triacylglyceride and alanine transaminase (ALT) concentrations were measured (Cobas 8000 modular analyser, Roche). Serum insulin concentrations were analysed (Immulite-1000, Siemens Healthcare Diagnostics). The Friedewald equation was used to calculate low-density lipoprotein (LDL) cholesterol concentrations (37). Glycated hemoglobin (HbA1c) concentrations were measured (fully automated HPLC Variant II, Bio-Rad Laboratories). In order to estimate the presence of insulin resistance, the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated out of fasting glucose and insulin concentrations in which $HOMA - IR = \frac{fastingglucose(\frac{mmol}{L}) \times fastinginsulin(\frac{mIU}{L})}{22.5}$ (38).

Blood pressure

Blood pressure (BP) was measured during daytime, every three minutes for about 20 times, depending on the tolerability of the child. (Mobil-O-Graph, I.E.M. GmbH, Stolberg, Germany). Mean systolic BP (SBP) and diastolic (DBP) were calculated and converted into percentiles using normative pediatric BP tables (39).

Polysomnography

All participants received a full polysomnography, and definitions of apnea and hypopnea were used as described in the appropriate manual (40). The apnea-hypopnea index (AHI) was calculated out of the average number of apneas and hypopneas per hour of total sleep time. The oxygen desaturation index (ODI) was calculated as the total of desaturations $\geq 3\%$ per hour.

Statistical analysis

Normally distributed data are presented as mean $\pm$ standard deviation, median (interquartile range) otherwise. In case of normally distributed data, Pearson's correlation coefficient was used, Spearman's correlation coefficient otherwise. A P-value below 0.05 was considered statistically significant, and all P-values are two-tailed. No correction was performed for multiple testing and P-values should be considered accordingly. The high sample size ($n = 600$) in this study may be responsible for turning clinically insignificant correlations into statistically significant correlations. As a rule of thumb, we here consider correlations < 0.200 as negligible from a clinical point of view. Statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Clinical Trail registration. ClinicalTrial.gov; Registration Number: NCT02091544.

RESULTS

Characteristics

In Table 3.1, anthropometrical and metabolic characteristics of the 600 included children are presented, stratified according to sex. Mean age was 12.20 ± 3.28 years (ranged from 2.61 to 17.88 years) and there were slightly more females compared to males. Mean BMI z-score was 3.31 ± 0.75 and 21.3% of the children was in the overweight category, and 44.7% and 34.0% in the obese and severe obese category.

Table 3.1. Clinical, anthropometrical and metabolic characteristics of the children.

Variable	Total (n=600)	Females (n = 321)	Males (n = 279)	P-value
Age (years)	12.20 ± 3.28	12.5 ± 3.4	11.9 ± 3.1	0.044*
Sex (%)	-	53.5	46.5	-
BMI z-score	3.31 ± 0.75	3.13 ± 0.70	3.52 ± 0.77	$<0.001^*$
BSA (m ²)	1.78 ± 0.43	1.77 ± 0.43	1.78 ± 0.43	0.849
Fat mass (%) ¹	44.07 ± 6.36	43.60 ± 6.36	44.55 ± 6.34	0.208
Waist circumference z-score	5.51 ± 2.28	5.39 ± 2.27	5.67 ± 2.28	0.150
Hip circumference z-score	4.04 ± 1.87	3.83 ± 1.79	4.28 ± 1.94	0.004*
Waist-to-hip ratio	0.92 ± 0.08	0.91 ± 0.08	0.95 ± 0.08	$<0.001^*$
SCr/Qage	0.99 ± 0.16	1.01 ± 0.15	0.97 ± 0.15	0.001*
SCr/Qage within [0.67 – 1.33]	96.5	96.0	97.1	0.508
SCr/Qheight	0.96 ± 0.17	0.98 ± 0.17	0.93 ± 0.15	0.001*
SCr/Qheight within [0.67 – 1.33]	94.7	93.8	95.7	0.294
Microalbuminuria (%) ²	6.7	8.7	4.4	0.070
Average SBP percentile ³	88 (73 - 96)	88 [71 – 96]	88 [73 -95]	0.641

Table 3.1. Clinical, anthropometrical and metabolic characteristics of the children. (continued)

Variable	Total (n=600)	Females (n = 321)	Males (n = 279)	P-value
Average DBP percentile ³	69 (50 - 84)	70 [51 - 84]	67 [49 - 83]	0.610
Fasting glucose (mmol/L)	4.31 ± 0.62	4.23 ± 0.64	4.40 ± 0.59	0.001 [*]
Fasting insulin (mU/L) ⁴	14.9 (9.4 - 22.7)	15.9 [10.7 - 23.8]	13.4 [8.2 - 20.8]	0.026 [*]
HOMA-IR ⁴	2.66 (1.72 - 4.23)	2.88 [1.83 - 4.29]	2.53 [1.48 - 4.19]	0.164
HbA1c (%)	5.26 ± 0.38	5.27 ± 0.39	5.25 ± 0.35	0.559
Total cholesterol (mmol/L)	4.3 ± 0.8	4.32 ± 0.79	4.21 ± 0.80	0.073
LDL-cholesterol (mmol/L)	2.5 ± 0.7	2.55 ± 0.73	2.43 ± 0.67	0.050
HDL-cholesterol (mmol/L)	1.3 ± 0.3	1.25 ± 0.29	1.28 ± 0.32	0.290
Triacylglyceride (mmol/L)	0.97 (0.71 - 1.34)	1.00 [0.74 - 1.37]	0.94 [0.66 - 1.30]	0.065
Uric acid (mg/dL) ⁵	5.08 (4.41 - 5.93)	5.08 (4.41 - 5.93)	5.08 (4.41 - 6.36)	0.065
ALT (U/L)	22 (17 - 31)	20.5 [16.0 - 27.0]	25.0 [19.0 - 36.0]	0.001 [*]
AHI ⁶	1.9 (1.0 - 3.8)	1.6 [0.8 - 3.3]	2.4 [1.2 - 4.6]	0.001 [*]
ODI ⁶	2.5 (1.1 - 4.4)	2.0 [0.9 - 3.6]	3.2 [1.4 - 5.4]	0.001 [*]

Data presented as mean ± standard deviation, median (interquartile range) and percentage (%). Abbreviations: BMI, body mass index; BSA, body surface area; SCr/Qage, serum creatinine normalized using Q-age polynomials; SCr/Qheight, serum creatinine normalized using Q-height polynomials; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine transaminase; AHI, apnea-hypopnea index; ODI, oxygen desaturation index. ¹ Data was available for 285 children (144 females, 141 males), percentage of total body weight. ² Data was available for 436 children (230 females, 206 males). ³ Based on a median of 21 (20-23) measurements, data was available for 554 children (291 females, 263 males). ⁴ Data was available for 416 children (226 females, 190 males). ⁵ Data was available for 388 children (195 females, 193 males). ⁶ Data was available for 450 children (245 females, 205 males). P-value is obtained with the two-sample t-test (continuous variables) or Fisher's exact test (binary variables).

In both sexes, mean BSA was about 1.78 m² and the body composed of approximately 44% fat mass. Compared to females, males had a significant higher BMI z-score, hip circumference z-score, waist-to-hip ratio, fasting glucose and alanine transaminase (ALT) concentration, and apnea hypopnea index (AHI) and oxygen desaturation index (ODI). 94.7% and 96.5% of the children had SCr/Qheight and SCr/Qage within the [0.67 - 1.33] reference interval, respectively. On the other hand, the concentration of fasting insulin was significantly higher in females and for triacylglyceride and LDL-cholesterol concentration there was a trend towards higher values in females compared to males.

As shown in Table 3.2, mean and median eGFR were 98.4 - 115.8 ml/min/1.73m² and 98.1 - 113.7 ml/min/1.73m² using the different creatinine-based GFR-estimating equations respectively. Moreover, equations differed concerning 2.5th and 97.5th percentile and range (minimum and maximum).

Table 3.2. Descriptive statistics of the performance of the different creatinine-based GFR-estimating equations.

	Mean	SD	Median	Percentile 2.5	Percentile 97.5	Minimum	Maximum	Age/ height based	Power coefficient
SCr/Qage	0.99	0.16	0.99	0.72	1.33	0.43	1.50	age	x
SCr/Qheight	0.96	0.17	0.94	0.66	1.31	0.55	1.69	height	x
eGFR (ml/ min/1.73m ²)									
FAS-age	111.0	18.3	108.5	80.7	149.6	71.5	252.4	age	-1
FAS-height	115.8	20.7	113.7	82.2	162.6	63.4	196.8	height	-1
EKFC	103.9	11.5	107.7	76.5	120.1	67.3	139.2	age	x
CKiD	113.7	20.1	111.4	79.1	159.1	66.1	189.1	height	-1
Schwartz-Lyon	103.4	18.0	101.5	72.1	141.7	58.9	186.0	height	-1
CKiDU25	106.5	18.2	104.2	76.7	144.8	64.2	226.6	height	-1
LMR18	98.4	11.4	98.1	75.3	120.2	67.0	144.4	age	x
CKD-EPI40	100.4	13.8	103.1	69.9	120.9	60.9	146.7	age	x

Abbreviations: SCr/Qage, serum creatinine normalized using Q-age polynomials; SCr/Qheight, serum creatinine normalized using Q-height polynomials; eGFR, estimated glomerular filtration rate; FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25 years, LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children.

In Supplementary Information Tables S3.1 and S3.2 anthropometrical and metabolic variables are compared between children with SCr/Qage and SCr/Qheight within the [0.67 – 1.33] reference range and values below 0.67 and above 1.33. As shown, groups are small and no multiple testing correction was performed, so the significance of the differences between the groups, should be interpreted with caution.

Correlation analyses

As presented in Table 3.3, SCr is correlated with age, weight, height, body surface area (BSA), fat mass, waist- and hip-circumference z-score and waist-to-hip ratio. In children between age of 2 and 14 years, however, SCr linearly increases with age with no differences between males and females. From the age of 14 years, SCr begins to differ between sexes and ends on a plateau value of 0.70 mg/dL for adult females and 0.90 mg/dL for adult males. So, since it is clear that SCr correlates with age and sex, SCr was rescaled using Q-age polynomials. Moreover, we rescaled SCr using Q-height polynomials as well. Correlations between SCr and age, weight, height, BSA and hip circumference z-score disappear when SCr is rescaled using Q-age. SCr/Qheight however is still correlated with weight, height and BSA. Correlations between SCr/Q (both Q-age and Q-height) and fat mass are becoming more pronounced, and there is still a correlation with waist circumference z-score and waist-to-hip ratio. SCr/Qheight was weakly inversely correlated with BMI z-score ($r = -0.109$, $P = 0.007$). SCr and SCr/Qage did not correlate with BMI z-score. All eGFR-equations correlated with fat mass and waist-to-hip ratio, whereas for the other anthropometric variables there was no consistency in correlations with eGFR.

In Table 3.4, correlations are shown between SCr, SCr/Q and different creatinine-based eGFR equations and metabolic parameters. SCr/Qage and nearly all eGFR-equations correlated with HOMA-IR and HDL-cholesterol, triacylglyceride, serum uric acid and ALT concentrations. Results are similar for SCr/Qheight with a few exceptions. Apart from this, there is a major inconsistency between eGFR-equations and (weak) correlations with metabolic variables.

Table 3.3. Correlations between SCr, SCr/Q and creatinine-based eGFR equations and clinical and anthropometric variables in children.

	Age (years)	Weight (kg)	Height (cm)	BMI z-score	BSA (m ²)	Fat mass (%) ¹	Waist circumference z-score	Hip circumference z-score	Waist-to-hip ratio
SCr (mg/dL)	0.732**	0.611**	0.688**	-0.037	0.649**	-0.139*	0.321**	0.445**	-0.237**
SCr/Qage	-0.034	-0.027	-0.001	-0.037	-0.023	-0.284**	-0.105*	0.017	-0.238**
SCr/Qheight	0.100*	-0.093*	-0.126*	-0.109*	-0.098*	-0.208**	-0.148**	-0.065	-0.207**
eGFR (ml/min/1.73 ²)									
FAS-age	0.047	0.037	0.004	0.040	0.031	0.296**	0.114*	-0.010	0.248**
FAS-height	-0.061	0.143*	0.160**	0.123*	0.144*	0.228**	0.189**	0.099*	0.212**
EKFC	-0.044	-0.039	-0.034	0.027	-0.036	0.262**	0.053	-0.072	0.258**
CKiD	-0.431**	-0.280**	-0.302**	0.088*	-0.295**	0.224**	-0.083*	-0.206**	0.240**
Schwartz-Lyon	-0.333**	-0.179**	-0.189**	0.098*	-0.189**	0.230**	-0.003	-0.117*	0.281**
CKiDU25	-0.107*	0.016	0.009	0.166**	0.013	0.267**	0.132*	0.037	0.290**
LMR18	-0.034	-0.027	-0.022	0.036	-0.024	0.292**	0.062	-0.061	0.280**
CKD-EPI40	-0.050	-0.041	-0.031	0.038	-0.037	0.272*	0.055	-0.068	0.277**

Data presented as Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient otherwise. Abbreviations: BMI, body mass index; BSA, body surface area; SCr, serum creatinine; SCr/Qage, serum creatinine normalized using Q-age polynomials; SCr/Qheight, serum creatinine normalized using Q-height polynomials;; eGFR, estimated glomerular filtration rate; FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25 years, LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children. ¹ Presented as percentage of the total body weight, data was available for 285 children. * P-value <0.05. ** P-value <0.001.

Table 3.4. Correlations between SCr, SCr/Q and creatinine-based GFR-estimating equations and metabolic variables in children.

	UACR (mg/g) ¹	Average SBP percentile ²	Average DBP percentile ²	Fasting glucose (mmol/L)	Fasting insulin (mU/L) ³	HOMA- IR ³	HbA1c (%)	Total cholesterol (mmol/L)	LDL- cholesterol (mmol/L)	HDL- cholesterol (mmol/L)	Triacyl- glyceride (mmol/L)	Serum uric acid (mg/ dL) ⁴	ALT (U/L)	AHI ⁵	ODI ⁵
SCr (mg/dL)	-0.054	-0.200**	-0.152**	0.001	0.112*	0.102*	-0.037	-0.032	-0.017	-0.137*	0.079	0.469**	0.039	0.011	-0.098
SCr/Qage	-0.071	-0.061	-0.082	-0.021	-0.135*	-0.114*	-0.076	-0.034	-0.034	0.102*	-0.122*	0.130*	-0.135*	-0.043	-0.064
SCr/Qheight	-0.098*	-0.050	0.000	-0.076	-0.131*	-0.137*	-0.090*	0.047	0.030	0.158**	-0.131*	0.081	-0.105*	0.019	0.013
eGFR (ml/ min/1.73m ²)															
FAS-age	0.112*	0.070	0.091*	0.026	0.145*	0.127*	0.072	0.037	0.038	-0.090*	0.112*	-0.147*	0.154*	0.070	0.095*
FAS-height	0.159*	0.050	0.0001	0.093*	0.149*	0.159*	0.086*	-0.047	-0.029	-0.165**	0.133*	-0.038	0.128*	-0.008	-0.002
EKFC	0.039	0.063	0.064	0.020	0.097*	0.076	0.073	0.015	0.014	-0.082*	0.094*	-0.147*	0.129*	0.052	0.065
CKiD	0.105*	0.148*	0.103*	0.028	0.015	0.019	0.058	0.0004	0.009	-0.023	0.026	-0.310**	0.043	-0.012	0.010
Schwartz- Lyon	0.101*	0.123*	0.099*	0.052	0.067	0.068	0.065	-0.016	-0.004	-0.060	0.047	-0.194**	0.102*	0.029	0.050
CKiDU25	0.103*	0.074	0.057	0.075	0.121*	0.118*	0.065	-0.014	-0.005	-0.096*	0.087*	-0.106*	0.183**	0.059	0.075
LMR18	0.055	0.073	0.080	0.039	0.111*	0.094	0.071	0.016	0.014	-0.080	0.098*	-0.150**	0.151**	0.061	0.080
CKD-EPI40	0.039	0.066	0.068	0.031	0.103*	0.084	0.073	0.014	0.015	-0.082*	0.094*	-0.138*	0.144*	0.063	0.078

Data presented as Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient otherwise. Abbreviations: UACR, urine albumin-to-creatinine ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine transaminase; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; SCr, serum creatinine; SCr/Qage, serum creatinine normalized using Q-age polynomials; SCr/Qheight, serum creatinine normalized using Q-height polynomials; eGFR, estimated glomerular filtration rate; FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25 years; LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children. ¹ Data was available for 436 children. ² Based on a median of 21 (20-23) measurements, data was available for 554 children. ³ Data was available for 416 children. ⁴ Data was available for 388 children. ⁵ Data was available for 450 children. * P-value <0.05. ** P-value <0.001.

DISCUSSION

In this study, we evaluated correlations between serum creatinine (SCr), rescaled SCr (SCr/Q) and different creatinine-based eGFR estimating equations with anthropometrical and metabolic variables in 600 children with overweight and obesity without overt kidney disease. SCr/Q-height correlated with BMI z-score, whereas SCr and SCr/Q-age did not. We verified the correlation between SCr and fat mass (and thus fat-free (lean) mass) (41), and this positive correlation becomes even more pronounced after rescaling SCr using Q-age and Q-height polynomials. Moreover, we showed that all examined creatinine-based eGFR-equations were positively correlated with fat mass and waist-to-hip ratio. Rescaled SCr and nearly all eGFR-equations correlated with HOMA-IR and HDL-cholesterol, triacylglyceride, serum uric acid and ALT concentrations. Apart from this, there is a major inconsistency between eGFR-equations and (weak) correlations with other metabolic variables. So, based on this study, it is clear that the choice of a creatinine-based eGFR-equation has an enormous impact on possible correlations with anthropometric variables and metabolic risk factors.

Obesity and excessive visceral fat are well known cardiovascular and metabolic risk factors. The cluster of metabolic, anthropometric and hemodynamic abnormalities are collectively known as metabolic syndrome. In children, there are at least 40 definitions of metabolic syndrome (42) and these definitions include parameters like waist-circumference, triacylglyceride, HDL-cholesterol and fasting glucose concentrations, and systolic blood pressure. In this study we evaluated these and other highly prevalent obesity-related comorbidities such as hypertension, altered glucose metabolism and insulin resistance, dyslipidemia, hyperuricemia, non-alcoholic fatty liver disease (NAFLD) and sleep-disordered breathing. SCr/Q and nearly all eGFR-equations correlated with HOMA-IR and HDL-cholesterol, triacylglyceride, serum uric acid and ALT concentrations; well-known markers for insulin resistance, dyslipidemia, hyperuricemia and NAFLD respectively. Our findings therefore support the relation between metabolic syndrome and the kidney in children with overweight and obesity (43).

This study also demonstrates that the choice of a creatinine-based eGFR-equation has a tremendous impact on correlations with the examined variables. Important to consider is that not all creatinine-based GFR-estimating equations seem suitable in children with overweight and obesity (19). Equations with SCr in the denominator (with '1' as power coefficient) may result in extremely high predictions. This is an artefact of these equations and exactly the reason why the EKFC equation has been developed. In this study, a difference in correlations with age-based and height-based creatinine-based eGFR-equations was observed. The CKiD, CKiDU25 and Schwartz-Lyon equations are the only equations that correlate significantly with age. Moreover, the 2.5th percentile is larger than 75 ml/min/1.73m² for nearly all equations (except for Schwartz-Lyon and

CKD-EPI40) and has previously been considered as the lower limit for GFR in children and adolescents (30). Finally, the EKFC, LMR18 and CKD-EPI40 equations show a much lower 'spread' (standard deviation) than the other equations, mainly because these equations use different power coefficients for SCr in their equation. This also results in much lower 97.5th percentile (and maximum) values.

Most other studies that examine the association between childhood obesity and kidney function use GFR-estimating equations or presence of microalbuminuria as markers for kidney function and body mass index (BMI) as measure for adiposity (13). In a recent review (13), 15 such studies were described and these studies found a mix of negative, positive and no associations between eGFR and BMI. In 10 studies, a Schwartz equation (most commonly the bedside Schwartz equation) was used for GFR-estimation, and in 9 of these studies a Schwartz equation was the only equation used. Though, the bedside Schwartz equation, that was developed and validated in growth-retarded children with CKD, shows a significant eGFR decline with age and major differences between adolescent males and females in children with a GFR within the normal range (44). Because of this, these Schwartz equations should not be used to examine associations between obesity and/or obesity-related comorbidities and kidney function in children without CKD.

All included studies in the review by Jadresic et al. used BMI as a marker for adiposity (13). While BMI is the most commonly used measure for obesity, it gives no information on body fat content or distribution. Human body mass can be divided into two main compartments: fat mass and lean (fat-free) mass. BMI does not distinguish lean mass from fat mass and it is known that the amount of adiposity correlates with cardiovascular risk factors independent from BMI (45). Several methods are currently available for body composition assessment and since field body composition methods (including anthropometrics, skinfolds, bio-electrical impedance analysis and ultrasound) are still inferior to more sophisticated laboratory body composition methods (46), air-displacement plethysmography was used in this study. Fat mass and waist-to-hip ratio, a surrogate marker for visceral fat, were correlated with SCr/Q and all examined creatinine-based GFR-estimating equations. These findings support the unhealthy aspect of visceral adiposity and suggests a relation between central adiposity and kidney function in children with overweight and obesity.

Due to the cross-sectional study design and lack of measured GFR, it is not possible to state which correlations are clinically relevant. Though, we suggest that rescaling SCr (either using Q-age or Q-height polynomials) can be a valuable add-on next to creatinine-based GFR-estimating equations.

Advantages of SCr/Q compared to eGFR-equations are:

- there is no influence of statistical modelling to convert SCr to an eGFR formula;
- there is a clear target value (namely $SCr/Q = 1$) for a healthy child, independent from age and sex, and a clear reference interval of [0.67 – 1.33];
- SCr/Q might be directly added to the serum creatinine result of a patient in the clinical laboratory report, as height is often unavailable in the clinical laboratory. In this manner interpretation of serum creatinine becomes more convenient for pediatricians;
- repeated measurements of SCr/Q over time are (probably) not influenced by age, sex and other factors (like body surface area (BSA));
- because SCr/Q is independent of BSA, it may serve as an excellent kidney function marker for longitudinal follow-up of children with overweight or obesity during a lifestyle program;
- SCr/Q correlates with fat-mass, waist-to-hip ratio, serum uric acid and ALT, variables that (almost) all examined eGFR-equations are correlated with in more or less the same degree.

The question whether childhood obesity is already related to or leads to future kidney disease can obviously not be answered in this cross-sectional study. While there are some examples of prospective studies that address these questions (12, 47-49), data on this are scarce. We suggest that future, prospective studies include SCr/Q as a kidney biomarker and compare our findings with measured GFR. Moreover, we suggest to include biomarkers for detection of early kidney damage due to obesity, such as urinary NGAL and KIM-1 (7).

In conclusion, correlations between obesity-related comorbidities and creatinine-based eGFR can be found in children with overweight and obesity, but depend to a large extent on the eGFR-equation of choice. SCr/Q is independent of the equation of choice and might be an alternative biomarker for assessing correlations between obesity-related comorbidities and kidney function in children with overweight and obesity.

REFERENCES

1. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, et al. (2017) Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 377:13-27
2. Skinner AC, Perrin EM, Moss LA, Skelton JA (2015) Cardiometabolic Risks and Severity of Obesity in Children and Young Adults. *N Engl J Med* 373:1307-1317
3. Juhola J, Magnussen CG, Viikari JS, Kähönen M, Hutri-Kähönen N, Jula A, Lehtimäki T, Åkerblom HK, Pietikäinen M, Laitinen T, Jokinen E, Taittonen L, Raitakari OT, Juonala M (2011) Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. *J Pediatr* 159:584-590
4. Weisinger JR, Kempson RL, Eldridge FL, Swenson RS (1974) The nephrotic syndrome: a complication of massive obesity. *Ann Intern Med* 81:440-447
5. Ejerblad E, Forel CM, Lindblad P, Fryzek J, McLaughlin JK, Nyrén O (2006) Obesity and risk for chronic renal failure. *J Am Soc Nephrol* 17:1695-1702
6. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, Praga M (2016) Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol* 12:453-471
7. Martínez-Montoro JL, Morales E, Cornejo-Pareja I, Tinahones FJ, Fernández-García JC (2022) Obesity-related glomerulopathy: Current approaches and future perspectives. *Obes Rev* 23:e13450
8. La Scola C, Guarino S, Pasini A, Capalbo D, Liguori L, Di Sessa A, Bertulli C, Mencarelli F, De Mutiis C, Campana G, La Manna A, Miraglia Del Giudice E, Pession A, Marzuillo P (2020) Effect of Body Mass Index on Estimated Glomerular Filtration Rate Levels in Children With Congenital Solitary Kidney: A Cross-Sectional Multicenter Study. *J Ren Nutr* 30:261-267
9. Bonnet F, Deprele C, Sassolas A, Moulin P, Alamartine E, Berthezène F, Berthouix F (2001) Excessive body weight as a new independent risk factor for clinical and pathological progression in primary IgA nephritis. *Am J Kidney Dis* 37:720-727
10. Nowak KL, You Z, Gitomer B, Brosnahan G, Torres VE, Chapman AB, Perrone RD, Steinman TI, Abebe KZ, Rahbari-Oskoui FF, Yu ASL, Harris PC, Bae KT, Hogan M, Miskulin D, Chonchol M (2018) Overweight and Obesity Are Predictors of Progression in Early Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol* 29:571-578
11. Bikbov B, Purcell CA, Levey AS et al. GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 395:709-733
12. Silverwood RJ, Pierce M, Hardy R, Thomas C, Ferro C, Savage C, Sattar N, Kuh D, Nitsch D (2013) Early-life overweight trajectory and CKD in the 1946 British birth cohort study. *Am J Kidney Dis* 62:276-284
13. Jadresic L, Silverwood RJ, Kinra S, Nitsch D (2019) Can childhood obesity influence later chronic kidney disease? *Pediatr Nephrol* 34:2457-2477
14. Stern-Zimmer M, Calderon-Margalit R, Skorecki K, Vivante A (2021) Childhood risk factors for adulthood chronic kidney disease. *Pediatr Nephrol* 36:1387-1396
15. Serra A, Romero R, Lopez D, Navarro M, Esteve A, Perez N, Alastrue A, Ariza A (2008) Renal injury in the extremely obese patients with normal renal function. *Kidney Int.* 73:947-955
16. Goknar N, Oktem F, Ozgen IT, Torun E, Kuçukkoc M, Demir AD, Cesur Y (2015) Determination of early urinary renal injury markers in obese children. *Pediatr Nephrol* 30:139-144
17. Mackowiak-Lewandowicz K, Ostalska-Nowicka D, Zaorska K, Kaczmarek E, Zachwieja J, Witt M, Nowicki M (2022) Chronic kidney disease predictors in obese adolescents. *Pediatr Nephrol* 37:2479-2488

18. Ding W, Mak RH (2015) Early markers of obesity-related renal injury in childhood. *Pediatr Nephrol* 30:1-4
19. van Dam MJCM, Pottel H, Vreugdenhil ACE (2022) Creatinine-based GFR-estimating equations in children with overweight and obesity. *Pediatr Nephrol* 37:2393-2403
20. Tsioufis C, Mazaraki A, Dimitriadis K, Stefanidis CJ, Stefanadis C (2011) Microalbuminuria in the paediatric age: current knowledge and emerging questions. *Acta Paediatr* 100:1180-1184
21. den Bakker E, Gemke R, Bokenkamp A (2018) Endogenous markers for kidney function in children: a review. *Crit Rev Clin Lab Sci* 55:163-183
22. van Dam M, Rijks J, Dorenbos E, Horuz F, van Dael K, Vreugdenhil A (2019) The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity. *Sci Rep* 9:4504
23. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 7:284-294
24. Fredriks AM, van Buuren S, Fekkes M, Verloove-Vanhorick SP, Wit JM (2005) Are age references for waist circumference, hip circumference and waist-hip ratio in Dutch children useful in clinical practice? *Eur J Pediatr* 164:216-222
25. Haycock GB, Schwartz GJ, Wisotsky DH (1978) Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr* 93:62-66
26. Pottel H, Vrydags N, Mahieu B, Vandewynckele E, Croes K, Martens F (2008) Establishing age/sex related serum creatinine reference intervals from hospital laboratory data based on different statistical methods. *Clin Chim Acta* 396:49-55
27. Ceriotti F, Boyd JC, Klein G, Henny J, Queraltó J, Kairisto V, Panteghini M (2008) Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem* 54:559-566
28. Pottel H, Hoste L, Martens F (2012) A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol* 27:973-979
29. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aïssa A, Cochat P, Martens F, Pottel H (2014) A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant* 29:1082-1091
30. Pottel H, Hoste L, Delanaye P (2015) Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m². *Pediatr Nephrol* 30:821-828
31. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, Dalton RN, Dubourg L, Gaillard F, Garrouste C, Grubb A, Jacquemont L, Hansson M, Kamar N, Lamb EJ, Legendre C, Littmann K, Mariat C, Melsom T, Rostaing L, Rule AD, Schaeffner E, Sundin PO, Turner S, Bökenkamp A, Berg U, Åsling-Monemi K, Selistre L, Åkesson A, Larsson A, Nyman U, Delanaye P (2021) Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate : A Cross-sectional Analysis of Pooled Data. *Ann Intern Med* 174:183-191
32. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL (2009) New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 20:629-637
33. De Souza VC, Rabilloud M, Cochat P, Selistre L, Hadj-Aïssa A, Kassai B, Ranchin B, Berg U, Herthelius M, Dubourg L (2012) Schwartz formula: is one k-coefficient adequate for all children? *PLoS One*. 7(12):e53439
34. Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ (2021) Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. *Kidney Int*. 99:948-956

35. Björk J, Nyman U, Delanaye P, Grubb A, Larsson A, Vranken L, Åkesson A, Pottel H (2020) A novel method for creatinine adjustment makes the revised Lund-Malmö GFR estimating equation applicable in children. *Scand J Clin Lab Invest* 80:456-463
36. Björk J, Nyman U, Larsson A, Delanaye P, Pottel H (2021) Estimation of the glomerular filtration rate in children and young adults by means of the CKD-EPI equation with age-adjusted creatinine values. *Kidney Int.* 99:940-947
37. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499-502
38. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419
39. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, de Ferranti SD, Dionne JM, Falkner B, Flinn SK, Gidding SS, Goodwin C, Leu MG, Powers ME, Rea C, Samuels J, Simasek M, Thaker VV, Urbina EM (2017) Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics*. 2017Sep;140(3):e20171904
40. Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Davidson Ward SL, Tangredi MM (2012) Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med* 8:597-619
41. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, Heilberg IP (2008) Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol* 3:348-354
42. Ford ES, Li C (2008) Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? *J Pediatr* 152:160-164
43. Litwin M, Niemirska A (2014) Metabolic syndrome in children with chronic kidney disease and after renal transplantation. *Pediatr Nephrol* 29:203-216
44. Pottel H, Björk J, Delanaye P, Nyman U (2022) Evaluation of the creatinine-based chronic kidney disease in children (under 25 years) equation in healthy children and adolescents. *Pediatr Nephrol* 37:2213-2216
45. Gishti O, Gaillard R, Durmus B, Abrahamse M, van der Beek EM, Hofman A, Franco OH, de Jonge LL, Jaddoe VW (2015) BMI, total and abdominal fat distribution, and cardiovascular risk factors in school-age children. *Pediatr Res* 77:710-718
46. Orsso CE, Silva MIB, Gonzalez MC, Rubin DA, Heymsfield SB, Prado CM, Haqq AM (2020) Assessment of body composition in pediatric overweight and obesity: A systematic review of the reliability and validity of common techniques. *Obes Rev* 21:e13041
47. Vivante A, Golan E, Tzur D, Leiba A, Tirosh A, Skorecki K, Calderon-Margalit R (2012) Body mass index in 1.2 million adolescents and risk for end-stage renal disease. *Arch Intern Med* 172:1644-1650
48. Silverwood RJ, Pierce M, Thomas C, Hardy R, Ferro C, Sattar N, Whincup P, Savage C, Kuh D, Nitsch D (2013) Association between younger age when first overweight and increased risk for CKD. *J Am Soc Nephrol* 24:813-821
49. Yan Y, Zheng W, Ma Q, Chu C, Hu J, Wang K, Liao Y, Chen C, Yuan Y, Lv Y, Xu X, Wang Y, Mu J (2021) Child-to-adult body mass index trajectories and the risk of subclinical renal damage in middle age. *Int J Obes (Lond)* 45:1095-1104

SUPPLEMENTARY INFORMATION

Comparison between children with SCr/Q within the [0.67 – 1.33] reference range, SCr/Q >1.33 and <0.67

94.7% and 96.5% of the 600 included children had a SCr/Qheight and SCr/Qage within the [0.67 – 1.33] reference interval, respectively. So, in only a minority of the children, SCr/Q was not within this reference range. While SCr/Q >1.33 (corresponding an eGFR-FAS of 81 ml/min/1.73m²) is indeed elevated, it still is debated whether an eGFR <75 ml/min/1.73m² (corresponding with a SCr/Q >1.43) should not be considered to be abnormal in children (1). Only 3 of the 600 children had a SCr/Qage >1.43 (values of 1.44, 1.49 and 1.50). Five children had a SCr/Qheight >1.43 (values of 1.45, 1.48, 1.51, 1.55 and 1.69).

In the following Supplementary Information Tables S3.1 and S3.2 anthropometrical and metabolic variables are compared between children with SCr/Qage and SCr/Qheight within the [0.67 – 1.33] reference range and values below 0.67 and above 1.33. As shown, groups are small and no multiple testing correction was performed, so the significance of the differences between the groups, should be interpreted with caution. Compared to children with a SCr/Q within the [0.67 – 1.33] reference range and children with a SCr/Q above 1.33, children with a SCr/Q below 0.67 have a higher fat mass percentage and so a lower lean body (and thus muscle) mass. Since a lower muscle mass is associated with a lower SCr, the low SCr/Q is likely to reflect low muscle mass in these children.

Supplementary Information Table S3.1. Clinical, anthropometrical and metabolic characteristics of the children stratified according to SCr/Qage within the [0.67 – 1.33] reference range, >1.33 or <0.67 respectively.

Variable	SCr/Qage within the [0.67 – 1.33] reference range (n=579)	SCr/Qage > 1.33 (n=14)	SCr/Qage < 0.67 (n=7)	P-value
Age (years)	12.2 [10.0 – 14.9]	11.3 [7.7 – 14.1]	14.1 [8.1 – 16.5]	0.455
Female sex (%)	53.2	71.4	42.9	0.341
BMI z-score	3.24 [2.79 – 3.81]	3.16 [2.54 – 3.57]	3.23 [2.65 – 3.65]	0.535
BSA (m ²)	1.77 [1.47 – 2.07]	1.73 [1.29 – 2.01]	1.91 [1.28 – 2.13]	0.522
Fat mass (%) ¹	44.3 [40.2 – 48.7]	40.5 [33.1 – 41.7]	60 [60 – 60]	0.063
Waist circumference z-score	5.20 [3.90 – 7.00]	4.05 [3.65 – 5.38]	6.00 [3.88 – 7.75]	0.120
Hip circumference z-score	3.90 [2.70 – 5.30]	3.25 [2.68 – 4.78]	4.25 [2.63 – 4.45]	0.356
Waist-to-hip ratio	0.92 [0.87 – 0.98]	0.88 [0.83 – 0.91]	1.01 [0.87 – 1.06]	0.018*
Microalbuminuria (%) ²	6.4	0.0	40.0	0.008*
Average SBP percentile ³	87 [72 – 96]	91 [75 – 97]	95 [80 – 99]	0.231
Average DBP percentile ³	68.5 [49.0 – 84.0]	75.0 [59.5 – 78.5]	80 [75 – 93]	0.139
Fasting glucose (mmol/L)	4.30 [3.90 – 4.70]	4.25 [4.00 – 4.53]	4.60 [3.90 – 4.90]	0.143
Fasting insulin (mU/L) ⁴	14.8 [9.4 – 22.7]	14.8 [7.4 – 20.0]	30.6 [10.9 – 69.1]	0.577
HOMA-IR ⁴	2.65 [1.73 – 4.22]	2.20 [1.38 – 4.04]	7.47 [2.08 – 15.49]	0.579
HbA1c (%)	5.25 [5.10 – 5.50]	5.15 [4.98 – 5.53]	5.15 [5.08 – 5.30]	0.630
Total cholesterol (mmol/L)	4.20 [3.70 – 4.80]	4.25 [3.60 – 4.80]	4.20 [3.80 – 5.20]	0.950
LDL-cholesterol (mmol/L)	2.40 [2.00 – 2.90]	2.50 [2.20 – 3.13]	2.30 [2.10 – 3.30]	0.952
HDL-cholesterol (mmol/L)	1.20 [1.05 – 1.40]	1.30 [0.98 – 1.63]	1.50 [1.00 – 1.60]	0.220
Triacylglyceride (mmol/L)	0.97 [0.71 – 1.34]	0.95 [0.68 – 1.22]	0.75 [0.48 – 1.37]	0.940
Uric acid (mg/dL) ⁵	5.08 (4.41 – 5.93)	5.00 [4.58 – 5.85]	5.42 [4.75 – 6.09]	0.437
ALT (U/L)	22.0 [17.0 – 30.8]	22.0 [16.5 – 28.5]	46.0 [18.0 – 74.0]	0.896

Supplementary Information Table S3.1. Clinical, anthropometrical and metabolic characteristics of the children stratified according to SCr/Qage within the [0.67 – 1.33] reference range, >1.33 or <0.67 respectively. (continued)

Variable	SCr/Qage within the [0.67 – 1.33] reference range (n=579)	SCr/Qage > 1.33 (n=14)	SCr/Qage < 0.67 (n=7)	P-value
AHI ⁶	1.9 [1.0 – 3.8]	1.85 [0.73 – 4.05]	7.25 [3.58 – 59.60]	0.118
ODI ⁶	2.5 [1.1 – 4.3]	1.70 [0.35 – 4.75]	9.45 [6.35 – 73.23]	0.117

Data presented as median (interquartile range) and percentage (%). Abbreviations: BMI, body mass index; BSA, body surface area; SCr/Qage, serum creatinine normalized using Q-age polynomials; SCr/Qheight, serum creatinine normalized using Q-height polynomials; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine transaminase; AHI, apnea-hypopnea index; ODI, oxygen desaturation index. ¹ Data was available for 285 children (only 1 child in the SCr/Qage <0.67 group), percentage of total body weight. ² Data was available for 436 children. ³ Based on a median of 21 (20-23) measurements, data was available for 554 children. ⁴ Data was available for 416 children. ⁵ Data was available for 388 children. ⁶ Data was available for 450 children. P-value is obtained with the Kruskal Wallis test or Fisher's exact test (binary variables). No multiple testing correction was performed.

Supplementary Information Table S3.2. Clinical, anthropometrical and metabolic characteristics of the children stratified according to SCr/Qheight within the [0.67 – 1.33] reference range, >1.33 or <0.67 respectively.

Variable	SCr/Qheight within the [0.67 – 1.33] reference range (n=568)	SCr/Qheight > 1.33 (n=13)	SCr/Qheight < 0.67 (=19)	P-value
Age (years)	12.1 [9.7 – 14.7]	15.6 [12.8 – 16.8]	14.3 [11.7 – 15.9]	0.010*
Female sex (%)	53.0	76.9	52.6	0.231
BMI z-score	3.24 [2.79 – 3.78]	3.32 [2.56 – 4.25]	3.24 [3.05 – 3.99]	0.867
BSA (m ²)	1.75 [1.45 – 2.06]	1.82 [1.60 – 2.06]	2.21 [2.07 – 2.61]	0.009*
Fat mass (%) ¹	43.9 [40.0 – 48.3]	41.0 [38.6 – 41.7]	48.5 [44.8 – 60.4]	<0.001*
Waist circumference z-score	5.10 [3.88 – 6.90]	4.30 [3.60 – 9.55]	7.00 [5.70 – 9.60]	0.009*
Hip circumference z-score	3.80 [2.65 – 5.15]	3.70 [2.85 – 5.65]	5.00 [4.20 – 6.50]	0.050
Waist-to-hip ratio	0.92 [0.87 – 0.98]	0.86 [0.83 – 0.95]	0.94 [0.87 – 1.06]	0.165
Microalbuminuria (%) ²	6.0	11.1	23.1	0.045*
Average SBP percentile ³	88 [73 – 96]	77 [69 – 96]	83.5 [71.5 – 95.3]	0.371
Average DBP percentile ³	69 [50 – 84]	75 [56 – 89]	59.0 [34.5 – 78.5]	0.866
Fasting glucose (mmol/L)	4.30 [3.90 – 4.70]	4.15 [3.93 – 4.45]	4.30 [4.00 – 5.25]	0.416
Fasting insulin (mU/L) ⁴	14.7 [9.2 – 22.3]	17.9 [10.0 – 27.3]	20.5 [11.5 – 44.5]	0.520
HOMA-IR ⁴	2.65 [1.71 – 4.19]	3.04 [1.84 – 5.21]	5.45 [2.09 – 10.90]	0.815
HbA1c (%)	5.20 [5.00 – 5.50]	5.40 [5.10 – 5.50]	5.20 [5.08 – 6.53]	0.451
Total cholesterol (mmol/L)	4.20 [3.70 – 4.80]	4.70 [4.05 – 5.20]	4.30 [3.60 – 4.80]	0.293
LDL-cholesterol (mmol/L)	2.40 [2.00 – 2.90]	3.00 [2.40 – 3.20]	2.30 [1.90 – 3.00]	0.186
HDL-cholesterol (mmol/L)	1.20 [1.10 – 1.40]	1.30 [1.05 – 1.50]	1.10 [1.00 – 1.30]	0.555
Triacylglyceride (mmol/L)	0.97 [0.71 – 1.34]	1.02 [0.80 – 1.41]	1.09 [0.87 – 1.37]	0.501
Uric acid (mg/dL) ⁵	5.08 (4.41 – 5.93)	5.76 [4.58 – 8.31]	5.42 [4.49 – 5.72]	0.591

Supplementary Information Table S3.2. Clinical, anthropometrical and metabolic characteristics of the children stratified according to SCr/Qheight within the [0.67 – 1.33] reference range, >1.33 or <0.67 respectively. (*continued*)

Variable	SCr/Qheight within the [0.67 – 1.33] reference range (n=568)	SCr/Qheight > 1.33 (n=13)	SCr/Qheight < 0.67 (=19)	P-value
ALT (U/L)	22.0 [17.0 – 30.0]	26.0 [20.5 – 33.0]	27.0 [18.0 – 52.0]	0.277
AHI ⁶	1.90 [1.00 – 3.80]	2.90 [0.70 – 11.28]	1.90 [0.90 – 5.00]	0.390
ODI ⁶	2.40 [1.10 – 4.40]	3.70 [0.68 – 10.90]	3.05 [1.23 – 5.28]	0.194

Data presented as median (interquartile range) and percentage (%). Abbreviations: BMI, body mass index; BSA, body surface

area; SCr/Qage, serum creatinine normalized using Q-age polynomials; SCr/Qheight, serum creatinine normalized using Q-height polynomials; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine transaminase; AHI, apnea-hypopnea index; ODI, oxygen desaturation index. ¹ Data was available for 285 children, percentage of total body weight. ² Data was available for 436 children. ³ Based on a median of 21 (20–23) measurements, data was available for 554 children. ⁴ Data was available for 416 children. ⁵ Data was available for 388 children. ⁶ Data was available for 450 children. P-value is obtained with the Kruskal Wallis test or Fisher's exact test (binary variables). No multiple testing correction was performed.

SUPPLEMENTARY REFERENCE

1. Pottel H, Hoste L, Delanaye P (2015) Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m². *Pediatr Nephrol* 30:821-828

4

Blood pressure in children with overweight and obesity: associations with estimated kidney function and metabolic aberrations

Mark J.C.M. van Dam, Hans Pottel, Anita C.E. Vreugdenhil

Not published

ABSTRACT

Background. The increased prevalence of hypertension in children is directly related with the childhood obesity pandemic. The aim of this study was to examine the anthropometric and metabolic characteristics of children within different blood pressure (BP) categories and to investigate whether office BP and estimated kidney function are correlated.

Methods. 554 children with overweight and obesity (52.5% female; mean age 12.29 ± 3.20 years; mean BMI z-score 3.29 ± 0.75) were included. All children received repeated office BP measurement, laboratory analysis and anthropometric evaluation.

Results. Based on a median of 21 (interquartile range 20-23) office BP measurements, 251 children (45.3%) had a normal BP measurement, 133 children (24.0%) an elevated BP measurement, 137 children (24.7%) stage 1 hypertensive BP measurement and 33 children (6.0%) stage 2 hypertensive BP measurement. Groups differed marginally though significantly regarding HDL-cholesterol, triacylglyceride and ALT concentration.

Conclusions. Hypertension is a common comorbidity in children with overweight and obesity, and office hypertensive BP measurement is present in about one in three children in our cohort. Compared to children with a normal office BP measurement, children with office hypertensive BP measurement were younger and demonstrated more dyslipidemia and slightly higher alanine aminotransferase (ALT) concentrations. Weak correlations between BP measurement and estimated kidney function were demonstrated.

INTRODUCTION

Arterial hypertension is a major global health issue and one of the main, potentially reversible, causes of cardiovascular disease worldwide. The prevalence of hypertension is estimated to be 3-5% in children. From puberty onwards, this prevalence increases and reaches 10-11% at the age of 18 years (1, 2). Childhood hypertension has long been considered as an uncommon condition, mostly secondary to renal, renovascular, endocrine or cardiac disorders. This situation has changed over the last few decades, since primary hypertension can present as early as 3 years of age and is nowadays thought to be the predominant form of hypertension in children older than 6 years (3). Elevated BP in children is associated with hypertension in adults (4).

The increased prevalence of primary hypertension in children is directly related with the increased prevalence of childhood obesity. The prevalence of primary hypertension in children with overweight and obesity was found to be 20 to 40%, depending on different reference values and the degree of adiposity (5). Primary hypertension was demonstrated to be the main comorbidity in children with overweight and obesity (6). Because of the strong association between adiposity and hypertension, in 2017 a revised guideline was published, with new normative pediatric BP tables based on normal-weight children (7).

Not all children with primary hypertension have adiposity and not all children with overweight or obesity have hypertension (2). Pathophysiology of both entities is complex and incompletely understood, but for obesity-related hypertension disturbed body composition and distribution of fat mass, increased sodium reabsorption and metabolic aberrations, like hyperinsulinemia and hyperuricemia, are likely to play a role (2, 8). There is major overlap between the pathogenesis of obesity-related hypertension and obesity-related chronic kidney disease (CKD) (8, 9).

The aim of this study was to examine the anthropometric and metabolic characteristics of children within different BP categories and to investigate whether office blood pressure and estimated kidney function are correlated.

METHODS

Setting and study inclusion

This single-centre, cross-sectional study was conducted within the clinical setting of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre (MUMC+) MosaKids Children's Hospital. Within COACH, children with overweight and obesity are offered extensive clinical evaluation and an outpatient multidisciplinary lifestyle intervention, as described previously (10). This study exclusively used baseline data, so before the lifestyle intervention started. All 662 participants who started participating in the COACH program between January 1st 2011 and April 1st 2019 were considered for inclusion in this study. Children with pre-existent kidney disease and/or current use of antihypertensive medication ($n=13$) were excluded. Moreover, children with normal weight or age >18 years at the moment of examinations ($n=10$) were excluded. Finally, since COACH is predominantly a clinical program, children (and/or their parents) could refuse blood analysis and/or repeated blood pressure measurement. Together with missing data and/or unreliable results, this has led to exclusion of another 85 children. Finally, 554 children were included in this study. Excluded children were slightly younger (mean age 11.04 ± 3.98 years) compared to included children. BMI z-score did not differ significantly between excluded and included children. The Medical Ethical Committee of the MUMC+ approved this study, and the guidelines administered by the Declaration of Helsinki were respected. Children (if ≥ 12 years) and their parents or legal guardian gave their informed consent.

Perinatal history, clinical assessment and anthropometry

From all children a detailed history was taken that also included perinatal and maternal factors such as gestational period, birth weight and length. From this, we determined whether a child was born small for gestational age (SGA) or not, defined as a birth weight and/or length below a standard deviation score of -2 . Besides, all children were subjected to a physical examination. Anthropometric data were collected according to relevant guidelines and regulations. Weight and height were measured and body mass index (BMI) and BMI z-score were determined. Overweight, obesity and severe obesity were defined according to the criteria of the International Obesity Task Force (IOTF) (11). Waist- and hip circumference and its ratio were determined. Fat mass and fat-free mass was determined by air displacement plethysmography (BodPod) in a subgroup of 285 children.

Blood pressure

Office (daytime) BP was measured approximately 20 times with an interval of three minutes between each measurement using an oscillometric BP device (Mobil-O-Graph, I.E.M. GmbH, Stolberg, Germany). The number of measurements could differ between children depending on personal tolerance; children should have had at least 3 consecutive BP measurements to be included in this study. The cuff size was fitted according to the

circumference of the upper arm according to the guideline (7). Mean systolic BP (SBP) and diastolic (DBP) was calculated and SBP and DBP percentiles were determined according to reference values for sex, age and height (7). BP variability was determined using the standard deviation (SD) and the coefficient of variation (CV; defined as the average SD of BP divided by the corresponding mean BP) (12). BP categories were defined using the following definitions (7): mean office BP was classified as 'normal' when mean BP was <90th percentile (for children aged 1-13 years) or when mean office BP was < 120/<80 mmHg (for children aged ≥ 13 years). Mean office BP was classified as 'elevated' when mean BP was ≥90th percentile to <95th percentile or 120/80 mmHg to <95th percentile (whichever was lower; for children aged 1-13 years), or when mean BP was 120/<80 to 129/<80 mmHg (for children aged ≥ 13 years). Stage 1 hypertensive measurement was defined as a mean office BP ≥ 95th percentile to <95th percentile + 12 mmHg, or 130/80 to 139/89 mmHg (whichever was lower; for children aged 1-13 years), or when mean office BP was 130/80 to 139/89 mmHg (for children aged ≥ 13 years). Stage 2 hypertensive measurement was defined as a mean office BP ≥ 95th + 12 mmHg, or ≥ 140/90 mmHg (whichever was lower; for children aged 1-13 years), or when mean office BP was ≥ 140/90 mmHg (for children aged ≥ 13 years).

Rescaled serum creatinine (SCr/Q) and estimated glomerular filtration rate (eGFR)

Serum creatinine (SCr) was measured using the enzymatic method (Cobas 8000, Roche). In order to adjust SCr for age and sex, or height, SCr was rescaled using reference Q-age and Q-height polynomials respectively (13, 14). Rescaled SCr (SCr/Q) was evaluated using the reference band of [0.67 – 1.33], representing the 2.5th and 97.5th percentile (15). SCr was converted into eGFR using the following equations: full-age spectrum (FAS), both height-independent (FAS-age) as height-dependent (FAS-height) (13, 14); new European Kidney Function Consortium (EKFC) (16); updated bedside Schwartz or CKiD (17); Schwartz-Lyon (18); CKiD under 25 years (CKiDU25) (19); adjusted-creatinine revised Lund-Malmö (LMR18) (20); and CKD-EPI equation with age-adjusted creatinine values (CKD-EPI40) (21).

Laboratory analyses

Venous blood samples were collected after an appropriate fasting state. Fasting plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, triacylglyceride, serum uric acid, alanine transaminase (ALT) and thyroid-stimulating hormone (TSH) concentrations were determined (Cobas 8000, Roche). Serum insulin concentrations were analysed (Immulite-1000, Siemens Healthcare Diagnostics). Glycated hemoglobin (HbA1c) concentrations were measured (fully automated HPLC Variant II, Bio-Rad Laboratories). The presence of insulin resistance was estimated with the homeostasis model assessment for insulin resistance (HOMA-IR) in which $HOMA-IR = \frac{fastingglucose(\frac{mmol}{L}) \times fastinginsulin(\frac{mIU}{L})}{22.5}$ (22). The Friedewald equation was used to calculate the low-density lipoprotein (LDL)

cholesterol concentrations (23). The quantity of microalbuminuria was estimated by means of a random urine spot. Albumin was measured using an immunoturbidimetric method and the urine albumin-to-creatinine ratio (UACR) was calculated by dividing the albumin concentration by the creatinine level. Microalbuminuria was defined as an UACR of ≥ 30 mg/g and ≤ 300 mg/g.

Statistical analysis

All statistics were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Normally distributed data are presented as mean $\pm$ standard deviation (SD), skewed data as median (interquartile range (IQR)). Comparisons between different groups were made with one-way ANOVA, Kruskal-Wallis test, Pearson's chi-square test and Fisher's exact test as appropriate. All p-values are two-tailed and a p-value below 0.05 was considered statistically significant. Clinical Trial registration. ClinicalTrial.gov; Registration Number: NCT02091544.

RESULTS

In Table 4.1 and 4.2 the characteristics of the 554 children are displayed. Mean age was 12.29 ± 3.20 years and 52.5% of the children were female. Mean BMI z-score was 3.29 ± 0.75 and 78% of the children had obesity or severe obesity. Based on median 21 (IQR 20-23) office blood pressure (BP) measurements, 251 children (45.3%) had a normal blood pressure (BP) measurement, 133 children (24.0%) an elevated BP measurement, 137 children (24.7%) stage 1 hypertensive BP measurement, and 33 children (6.0%) stage 2 hypertensive BP measurement. 94.8% and 96.4% of the children had SCr/Qheight and SCr/Qage within the [0.67 - 1.33] reference interval, respectively. Mean eGFR varied between 98.4 - 115.7 ml/min/1.73m², depending on GFR equation. 6.8% of the children had microalbuminuria.

Stratification according to office BP classification

In Table 4.1 and 4.2, the characteristics of the children stratified according to office BP classification are displayed. Compared to children with normal and elevated BP measurement, children with office hypertensive BP measurement were significantly younger and tend to have a higher BMI z-score. Groups differed marginally though significantly regarding SD of SBP, SD of DBP, and HDL-cholesterol, triacylglyceride and ALT concentration. As displayed in Table 4.1, between the groups, no significant differences concerning sex, being born SGA, fat-mass percentage of total body weight, percentage of microalbuminuria, and serum uric acid concentration were demonstrated. Moreover, no significant differences were demonstrated between BP categories and SCr/Q or eGFR. In order to examine the possible correlation between BP and SCr/Q-age and SCr/Q-height,

we performed a post hoc Tukey's range test; no significant differences between the groups could be demonstrated.

Correlation analyses

So based on the ANOVA and post hoc Tukey's range test, no significant differences between BP categories and estimated kidney function could be demonstrated. In order to examine if estimated kidney function and BP were somehow related, correlation analyses was performed. Mean office SBP percentile was weakly, though significantly, correlated with eGFR CKiD ($r = 0.147, p < 0.001$) and eGFR Schwartz-Lyon ($r = 0.122, p = 0.004$). Mean office DBP percentile was weakly, though significantly, correlated with SCr/Q-age ($r = -0.104, p = 0.015$), eGFR FAS-age ($r = 0.104, p = 0.015$), eGFR EKFC ($r = 0.091, p = 0.033$), eGFR CKiD ($r = 0.112, p = 0.008$), eGFR Schwartz-Lyon ($r = 0.105, p = 0.014$), eGFR LMR18 ($r = 0.088, p = 0.0388$) and eGFR CKD-EPI40 ($r = 0.0887, p = 0.0368$). SCr/Q-height (and thus eGFR FAS-height) was not correlated with BP.

Table 4.1. Characteristic of the children with overweight and obesity, stratified according to classification of the office blood pressure (BP) measurements.

Variable	Total (n=554)	Normal BP measurement (n=251)	Elevated BP measurement (n=133)	Stage 1 hypertensive BP measurement (n=137)	Stage 2 hypertensive BP measurement (n=33)	Stage 1 and 2 hypertensive BP measurement combined (n=170)	
						P-value ^a	P-value ^b
Age (years)	12.29 ± 3.20	12.61 ± 2.93	12.33 ± 3.31	11.66 ± 3.29	12.44 ± 4.00	0.048*	0.042*
Male/female (%)	47.5 / 52.5	45.4 / 54.6	51.1 / 48.9	47.4 / 52.6	48.5 / 51.5	0.765	0.566
SGA (%)	8.5	8.8	7.5	8.8	9.1	0.951	0.841
BMI z-score	3.29 ± 0.75	3.21 ± 0.75	3.30 ± 0.80	3.39 ± 0.66	3.39 ± 0.81	0.114	0.051
Overweight/obesity/severe obesity (%)	22 / 44 / 34	27 / 41 / 32	22 / 47 / 31	15 / 47 / 38	15 / 46 / 39	0.106	0.034*
Waist-to-hip ratio	0.92 ± 0.08	0.92 ± 0.08	0.92 ± 0.09	0.93 ± 0.08	0.94 ± 0.07	0.233	0.123
Fat mass (% of total weight) ¹	44.2 ± 6.3	44.5 ± 6.5	44.6 ± 5.9	43.9 ± 6.1	42.5 ± 7.4	0.585	0.541
Number of BP measurements	21 (20 - 23)	21 (19 - 23)	22 (20 - 23)	21 (20 - 23)	22 (20 - 23)	0.004*	0.002*
SD of SBP	9.2 ± 3.4	8.7 ± 3.2	9.4 ± 3.7	9.9 ± 3.5	9.7 ± 2.9	0.006*	0.002*
SBP coefficient of variation	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.07 ± 0.02	0.327	0.870
SD of DBP	6.9 ± 2.2	6.7 ± 2.0	6.8 ± 2.3	7.3 ± 2.2	7.7 ± 2.3	0.008*	0.005*
DBP coefficient of variation	0.10 ± 0.03	0.11 ± 0.04	0.10 ± 0.04	0.10 ± 0.03	0.10 ± 0.03	0.354	0.197
Microalbuminuria (%) ²	6.8	6.6	6.9	8.1	3.7	0.879	0.982
Fasting plasma glucose (mmol/L)	4.3 ± 0.6	4.3 ± 0.6	4.3 ± 0.6	4.3 ± 0.6	4.5 ± 0.6	0.522	0.864
Fasting insulin (mU/L) ³	14.9 (9.4 - 22.7)	14.2 (8.6 - 22.0)	15.9 (10.0 - 22.0)	14.3 (9.5 - 23.8)	21.3 (12.4 - 33.0)	0.091	0.314
HOMA-IR ³	2.7 (1.7 - 4.2)	2.5 (1.5 - 4.1)	2.8 (1.9 - 4.2)	2.8 (1.7 - 4.3)	4.5 (2.3 - 6.9)	0.106	0.321
Total cholesterol (mmol/L)	4.3 ± 0.8	4.2 ± 0.8	4.4 ± 0.8	4.3 ± 0.8	4.3 ± 0.7	0.149	0.070
LDL-cholesterol (mmol/L)	2.5 ± 0.7	2.4 ± 0.7	2.5 ± 0.7	2.6 ± 0.7	2.5 ± 0.7	0.193	0.097
HDL-cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.050	0.023*
Triacylglyceride (mmol/L)	0.97 (0.71 - 1.34)	0.92 (0.66 - 1.21)	1.00 (0.76 - 1.30)	1.00 (0.72 - 1.45)	1.04 (0.85 - 1.61)	0.017*	0.011*

Table 4.1. Characteristic of the children with overweight and obesity, stratified according to classification of the office blood pressure (BP) measurements.
(continued)

Variable	Total (n=554)	Normal BP measurement (n=251)	Elevated BP measurement (n=133)	Stage 1 hypertensive BP measurement (n=137)	Stage 2 hypertensive BP measurement (n=33)	Stage 1 and 2 hypertensive BP measurement combined (n=170)	P-value ^a	P-value ^b
Serum uric acid (mg/dL) ⁴	5.3 ± 1.3	5.2 ± 1.3	5.4 ± 1.2	5.3 ± 1.2	5.7 ± 1.7	5.3 ± 1.4	0.459	0.750
ALT (U/L)	22 (17 - 31)	21 (16 - 29)	24 (19 - 32)	23 (18 - 31)	23 (19 - 43)	23 (18 - 33)	0.053	0.046*
TSH (mU/L)	3.0 ± 1.3	3.0 ± 1.2	3.0 ± 1.3	3.1 ± 1.5	2.7 ± 1.4	3.1 ± 1.5	0.394	0.853

Data presented as mean ± standard deviation or median (interquartile range). P-value obtained from one-way ANOVA, Kruskal-Wallis test, Pearson's chi-square test and Fisher's exact test as appropriate to compare the groups of normal BP measurement, elevated BP measurement, stage 1 hypertensive BP measurement, and stage 2 hypertensive BP measurement (^a) or the groups of normal BP measurement, elevated BP measurement, and stage 1 and stage 2 hypertensive BP measurement combined (^b). ¹ Data was available for 285 children (144 females, 141 males). ² Data was available for 436 children (230 females, 206 males). ³ Data was available for 416 children (226 females, 190 males). ⁴ Data was available for 388 children (195 females, 193 males). Abbreviations: SGA, small for gestational age; BMI, body mass index; BP, blood pressure; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine transaminase; TSH, thyroid stimulating hormone.

Table 4.2. Rescaled serum creatinine (SCr/Q) and estimated glomerular filtration rate (eGFR) of the children with overweight and obesity, stratified according to classification of the office blood pressure (BP) measurements.

Variable	Total (n=554)	Normal BP measurement (n=251)	Elevated BP measurement (n=133)	Stage 1 hypertensive BP measurement (n=137)	Stage 2 hypertensive BP measurement (n=33)	P-value ^a	Stage 1 and 2 hypertensive BP measurement combined (n=170)	P-value ^b
SCr/Q-age	0.99 ± 0.16	0.99 ± 0.15	1.00 ± 0.16	1.00 ± 0.16	0.94 ± 0.16	0.181	0.99 ± 0.16	0.587
SCr/Q-age within [0.67 – 1.33]	96.4%	97.6%	96.2%	94.9%	93.9%	0.469	94.7%	0.291
SCr/Q-height	0.96 ± 0.17	0.96 ± 0.17	0.97 ± 0.16	0.95 ± 0.16	0.89 ± 0.15	0.156	0.94 ± 0.16	0.334
SCr/Q-height within [0.67 – 1.33]	94.8%	93.6%	95.5%	96.4%	93.9%	0.674	95.9%	0.542
eGFR FAS-age	111.0 ± 18.5	111.2 ± 18.5	109.7 ± 17.7	110.4 ± 18.2	117.8 ± 21.1	0.150	111.8 ± 19.0	0.591
eGFR FAS-height	115.7 ± 20.9	115.4 ± 21.7	114.2 ± 20.1	116.0 ± 19.7	123.5 ± 22.6	0.148	117.5 ± 20.4	0.379
eGFR EKFC	103.9 ± 11.5	104.3 ± 11.1	103.0 ± 12.1	103.5 ± 11.8	106.5 ± 11.0	0.383	104.0 ± 11.7	0.554
eGFR CKiD ('bedside Schwartz')	113.4 ± 20.1	113.0 ± 19.1	111.4 ± 18.8	114.8 ± 22.2	118.1 ± 23.6	0.280	115.5 ± 22.4	0.206
eGFR Schwartz-Lyon	103.3 ± 18.0	102.7 ± 17.6	101.9 ± 16.8	104.2 ± 19.2	109.0 ± 20.3	0.193	105.2 ± 19.5	0.241
eGFR CKiD-U25	106.5 ± 18.3	106.1 ± 18.4	105.5 ± 17.2	106.7 ± 18.5	113.2 ± 20.5	0.173	108.0 ± 19.0	0.434
eGFR LMR18	98.4 ± 11.4	98.7 ± 11.2	97.6 ± 11.4	98.1 ± 11.7	101.4 ± 11.7	0.343	98.7 ± 11.7	0.603
eGFR CKD-EPI40	100.4 ± 13.8	100.7 ± 13.5	99.6 ± 14.3	99.8 ± 14.0	103.6 ± 13.3	0.460	100.6 ± 13.9	0.730

Data presented as mean ± standard deviation. P-value obtained from one-way ANOVA, Pearson's chi-square test and Fisher's exact test as appropriate to compare the groups of normal BP measurement, elevated BP measurement, stage 1 hypertensive BP measurement, and stage 2 hypertensive BP measurement ^(a) or the groups of normal BP measurement, elevated BP measurement, and stage 1 and stage 2 hypertensive BP measurement combined ^(b). Abbreviations: BP, blood pressure; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials; FAS, full-age spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25; LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children.

DISCUSSION

In this study, we examined office blood pressure (BP) in a cohort of 554 children with overweight and obesity. Using the revised normal BP references for children (7) and based on a median of 21 (IQR 20-23) office measurements, less than half of the children had a normal BP measurement, and 24%, 25% and 6.0% of the children had elevated BP measurement, stage 1 hypertensive BP measurement, and stage 2 hypertensive BP measurement, respectively. So, in this study, the prevalence of office hypertensive BP measurement was 31%. Compared to children with a normal office BP measurement, children with office hypertensive BP measurement were younger, tend to have a higher BMI z-score, and demonstrated more dyslipidemia and slightly higher alanine aminotransferase (ALT) concentrations. Contrary to our expectations, the group of children with office hypertensive BP measurement did not display significant more insulinemia or hyperuricemia compared to children without office hypertensive BP measurement. Moreover, children with office hypertensive BP measurement did not have significantly different estimated kidney function compared to children without office hypertensive BP measurement.

Hypertension is a common cardiovascular risk factor in children with overweight and obesity and rates of hypertension increase in a graded fashion with increasing adiposity (24, 25). The prevalence of primary hypertension in children with overweight and obesity was found to be 20 to 40%, heavily depending on different reference values and the degree of adiposity (5). Childhood obesity is also related with the development of future hypertension (4, 26). In a school-based screening among children (mean age 13.5 ± 1.7 years), the prevalence of hypertension increased with BMI and adjusted relative risk (RR) of hypertension was significant for overweight (RR 3.26, confidence interval (CI) 2.50-4.24) and being male (RR 1.50, CI 1.15-1.95) (27). In another study (28), describing an Icelandic school cohort of 970 children (mean age 9.6 ± 0.3 years, prevalence of overweight and obesity 20.1 and 7.2% respectively), the prevalence of hypertension was 2.5% and BP was correlated with BMI. Prevalence of hypertension did not differ between sexes in this study. In another study among 1111 children (mean age 10.2 years, range 4-17 years) (29) it was found that the effect of adiposity on BP was modest (and not statistically significant) until BMI reached the 90th percentile at which point the effect of BMI on BP became significant; odds ratio (OR) of BMI on prehypertension (nowadays called elevated BP) and hypertension was 1.11 (95% CI 1.08-1.14). A large study from Europe (57,915 children, aged 6 to 18 years) demonstrated a higher prevalence of hypertension in males compared to females and in children with obesity and severe obesity compared to children with overweight) (5); this study also underlined the large effects of using different national or international BP references.

Diagnosing hypertension in children with overweight and obesity can be difficult and its prevalence is highly dependent on how BP is measured and interpreted. This is evidenced by a study from the Netherlands (30) on 1407 children (median age 11.7 years, 49% boys, 969 children with overweight and obesity and 438 lean children) in which the lowest of three BP measurements on two occasions was compared with the first and mean of two measurements on one occasion. The prevalence of hypertension ranged from 4.4% (when the lowest of three BP measurements was used) to 33%. The importance of repeated measurements is underscored by other studies as well (27, 28), in which the prevalence of hypertension in children decreases with each visit.

Obesity-associated risk factors that promote hypertension include higher age, disturbed lipid metabolism, hyperinsulinemia, hyperuricemia, excess sodium intake and physical inactivity. Besides this, obesity leads to hypertension via multiple mechanisms, such as increased activity of the renin-angiotensin-aldosterone system and sympathetic nervous system, and resulting sodium and fluid retention (8). In our study, children with office hypertensive BP measurement did indeed demonstrate more dyslipidemia, including lower HDL-cholesterol and higher triacylglyceride concentrations, compared to children with normal office BP measurement. Although higher insulin levels and insulin resistance were found to precede the development of primary hypertension by others (31), in our study, children with and without office hypertensive BP measurement did not significantly differ in insulinemia or HOMA-IR. Moreover, in our study, also contrary to our expectations, children with and without office hypertensive BP measurement did not differ in serum uric acid concentration, while other studies found that serum uric acid was independently associated with BP levels (32).

The relation between obesity, hypertension and chronic kidney disease (CKD) is well established in adults. Obesity in adulthood is independently associated with incident CKD and CKD progression (9) and it is likely that childhood obesity is related with CKD later in life (33), possibly via accelerating hypertension. Hypertension in otherwise healthy adolescents is associated with a doubling of the risk of future end-stage kidney disease (34), though, it is unclear whether this is a causal relationship. Considering the overlap in pathogenic alterations that lead to hypertension and CKD in the context of obesity (like increased sodium reabsorption and metabolic aberrations), we hypothesized that hypertension and kidney function are already associated in children with overweight and obesity. As described before, most children in our cohort have a SCr within the normal reference range for children (35) and it is known that the choice of SCr-based eGFR equation has an enormous impact on the value of eGFR (36). Therefore, rescaled SCr (SCr/Q) was used in this study. While we could demonstrate some weak but significant univariate correlations between estimated kidney function (SCr/Q-age and multiple eGFR equations) and BP percentile, no clear correlations were found between estimated kidney function and BP.

Being born small for gestational age (SGA) is both a risk factor for obesity and for the development of hypertension and CKD (37), this latter explained by the fact that individuals with a congenital nephron deficit (which is the case in children born SGA) have less reserve capacity in kidney function. In our study, we could not demonstrate a correlation between BP and being born SGA.

This study is not without limitations. First, an automated oscillometric device, as opposed to auscultatory measurements, was used to determine BP. Although normative BP values are based on auscultatory measurements, oscillometric BP devices have become common in clinical settings (38). Compared to auscultatory measurements, BP determined by oscillometric devices however tend to overestimate BP and the within-visit variation of the BP is larger (7). Second, BP measurements were conducted during the same day, whereas multiple BP measurements over time is advised (7). In order to decrease bias caused by too little measurements, in this study BP was measured a median of 21 times (interquartile range 20-23); which is inferior to repeated measurements over time. Third, this study did not include ABPM, the gold standard measurement to diagnose hypertension. Whilst office measurements are used in daily practice, low agreement was found between both automated-devices or manual obtained BP and ABPM (39, 40). Moreover, since ABPM was not included, we could not identify children with white coat hypertension or masked hypertension, the latter is defined as an office BP <95th percentile and mean ambulatory SBP or DBP >95th percentile and elevated SBP or DBP load (>25%). Masked hypertension is common in children with overweight and obesity (41) and is an independent risk factor for end-organ hypertensive damage (42). Fourth, we did not include markers for vascular or cardiac dysfunction. Fifth, we did not objectify daily salt and sugar intake, both important obesity-related risk factors for hypertension (43) nor physical activity in this study. Finally, kidney function was estimated with serum creatinine (SCr) and we did not include other endogenous markers such as cystatin C, nor did we include measured GFR.

In conclusion, hypertension is a common comorbidity in children with overweight and obesity, and office hypertensive BP measurement is present in about one in three children in our cohort. Compared to children with a normal office BP measurement, children with office hypertensive BP measurement were younger and demonstrated more dyslipidemia and slightly higher alanine aminotransferase (ALT) concentrations. Weak correlations between BP measurement and estimated kidney function were demonstrated.

REFERENCES

1. Song P, Zhang Y, Yu J, Zha M, Zhu Y, Rahimi K, et al. Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr.* 2019;173(12):1154-63
2. Litwin M, Kułaga Z. Obesity, metabolic syndrome, and primary hypertension. *Pediatr Nephrol.* 2021;36(4):825-37
3. Gupta-Malhotra M, Banker A, Shete S, Hashmi SS, Tyson JE, Barratt MS, et al. Essential hypertension vs. secondary hypertension among children. *Am J Hypertens.* 2015;28(1):73-80
4. Azegami T, Uchida K, Tokumura M, Mori M. Blood Pressure Tracking From Childhood to Adulthood. *Front Pediatr.* 2021;9:785356
5. Flechtner-Mors M, Neuhauser H, Reinehr T, Roost HP, Wiegand S, Siegfried W, et al. Blood pressure in 57,915 pediatric patients who are overweight or obese based on five reference systems. *Am J Cardiol.* 2015;115(11):1587-94
6. Flechtner-Mors M, Thamm M, Wiegand S, Reinehr T, Schwab KO, Kiess W, et al. Comorbidities related to BMI category in children and adolescents: German/Austrian/Swiss Obesity Register APV compared to the German KiGGS Study. *Horm Res Paediatr.* 2012;77(1):19-26
7. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics.* 2017;140(3)
8. Wühl E. Hypertension in childhood obesity. *Acta Paediatr.* 2019;108(1):37-43
9. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol.* 2016;12(8):453-71
10. van Dam M, Rijks J, Dorenbos E, Horuz F, van Dael K, Vreugdenhil A. The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity. *Sci Rep.* 2019;9(1):4504
11. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes.* 2012;7(4):284-94
12. Parati G, Ochoa JE, Lombardi C, Bilo G. Assessment and management of blood-pressure variability. *Nat Rev Cardiol.* 2013;10(3):143-55
13. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol.* 2012;27(6):973-9
14. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aïssa A, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant.* 2014;29(5):1082-91
15. Pottel H, Hoste L, Delanaye P. Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m(2). *Pediatr Nephrol.* 2015;30(5):821-8
16. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, et al. Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate: A Cross-sectional Analysis of Pooled Data. *Ann Intern Med.* 2021;174(2):183-91
17. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol.* 2009;20(3):629-37
18. De Souza VC, Rabilloud M, Cochat P, Selistre L, Hadj-Aïssa A, Kassai B, et al. Schwartz formula: is one k-coefficient adequate for all children? *PLoS One.* 2012;7(12):e53439
19. Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ. Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. *Kidney Int.* 2021;99(4):948-56

20. Björk J, Nyman U, Delanaye P, Grubb A, Larsson A, Vranken L, et al. A novel method for creatinine adjustment makes the revised Lund-Malmö GFR estimating equation applicable in children. *Scand J Clin Lab Invest*. 2020;80(6):456-63
21. Björk J, Nyman U, Larsson A, Delanaye P, Pottel H. Estimation of the glomerular filtration rate in children and young adults by means of the CKD-EPI equation with age-adjusted creatinine values. *Kidney Int*. 2021;99(4):940-7
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-9
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499-502
24. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic Risks and Severity of Obesity in Children and Young Adults. *N Engl J Med*. 2015 Oct;373(14):1307-17
25. Friedemann C, Heneghan C, Mahtani K, Thompson M, Perera R, Ward AM. Cardiovascular disease risk in healthy children and its association with body mass index: systematic review and meta-analysis. *BMJ*. 2012 Sep 25;345:e4759
26. Parker ED, Sinaiko AR, Kharbanda EO, Margolis KL, Daley MF, Trower NK, et al. Change in Weight Status and Development of Hypertension. *Pediatrics*. 2016;137(3):e20151662
27. Sorof JM, Lai D, Turner J, Poffenbarger T, Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics*. 2004;113(3 Pt 1):475-82
28. Steinhorsdottir SD, Eliasdottir SB, Indridason OS, Agustsdottir IM, Palsson R, Edvardsson VO. Prevalence of hypertension in 9- to 10-year-old Icelandic school children. *J Clin Hypertens (Greenwich)*. 2011 Oct;13(10):774-9
29. Tu W, Eckert GJ, DiMeglio LA, Yu Z, Jung J, Pratt JH. Intensified effect of adiposity on blood pressure in overweight and obese children. *Hypertension*. 2011;58(5):818-24
30. Wirix AJ, Nauta J, Groothoff JW, Rabelink TJ, HiraSing RA, Chinapaw MJ, et al. Is the prevalence of hypertension in overweight children overestimated? *Arch Dis Child*. 2016;101(11):998-1003
31. Srinivasan SR, Myers L, Berenson GS. Changes in metabolic syndrome variables since childhood in prehypertensive and hypertensive subjects: the Bogalusa Heart Study. *Hypertension*. 2006;48(1):33-9
32. Goli P, Riahi R, Daniali SS, Pourmirzaei M, Kelishadi R. Association of serum uric acid concentration with components of pediatric metabolic syndrome: A systematic review and meta-analysis. *J Res Med Sci*. 2020;25:43
33. Jadresic L, Silverwood RJ, Kinra S, Nitsch D. Can childhood obesity influence later chronic kidney disease? *Pediatr Nephrol*. 2019;34(12):2457-77
34. Leiba A, Fishman B, Twig G, Gilad D, Derazne E, Shamiss A, et al. Association of Adolescent Hypertension With Future End-stage Renal Disease. *JAMA Intern Med*. 2019;179(4):517-23
35. van Dam M, Pottel H, Vreugdenhil ACE. Creatinine-based GFR-estimating equations in children with overweight and obesity. *Pediatr Nephrol*. 2022;37(10):2393-403
36. van Dam M, Pottel H, Vreugdenhil ACE. Relation between obesity-related comorbidities and kidney function estimation in children. *Pediatr Nephrol*. 2023;38(6):1867-76
37. Luyckx VA, Brenner BM. Low birth weight, nephron number, and kidney disease. *Kidney Int Suppl*. 2005(97):S68-77
38. Alpert BS, Quinn D, Gallick D. Oscillometric blood pressure: a review for clinicians. *J Am Soc Hypertens*. 2014;8(12):930-8

39. Hanevold CD, Faino AV, Flynn JT. Use of Automated Office Blood Pressure Measurement in the Evaluation of Elevated Blood Pressures in Children and Adolescents. *J Pediatr.* 2020;227:204-11.e6
40. van der Heijden LB, Groothoff JW, Feskens EJ, Janse AJ. Office blood pressure versus ambulatory blood pressure measurement in childhood obesity. *BMC Pediatr.* 2023;23(1):205
41. Aguilar A, Ostrow V, De Luca F, Suarez E. Elevated ambulatory blood pressure in a multi-ethnic population of obese children and adolescents. *J Pediatr.* 2010;156(6):930-5
42. Stabouli S, Kotsis V, Tzoumanidis S, Papamichael C, Constantopoulos A, Zakopoulos N. White-coat and masked hypertension in children: association with target-organ damage. *Pediatr Nephrol.* 2005;20(8):1151-5
43. Genovesi S, Giussani M, Orlando A, Orgiu F, Parati G. Salt and Sugar: Two Enemies of Healthy Blood Pressure in Children. *Nutrients.* 2021;13(2)

5

Real-time continuous glucose monitoring and estimated kidney function in children with overweight and obesity

Mark J.C.M. van Dam, Hans Pottel, Anita C.E. Vreugdenhil

Not published

ABSTRACT

Introduction. Obesity is an independent risk factor for the development of chronic kidney disease (CKD). Hyperglycemia is known to play an important role in the development of glomerular hyperfiltration, the initial phase of CKD in the setting of both obesity and diabetes. The aim of this study was to examine the relationship between the glycemic profile and estimated kidney function in children with overweight and obesity.

Methods. This cross-sectional study included 106 children with overweight and obesity without diabetes (mean age 12.37 ± 2.87 years, 60.4% females, mean BMI z-score 3.42 ± 0.69). Venous blood sampling was performed after which an oral glucose tolerance test was performed. All children underwent 48 hour real-time continuous glucose monitoring (rtCGM).

Results. Of the children, 56.6% had a HOMA-IR >2.5 (suggesting insulin resistance), 3.8% and 23.6% of the children had impaired glucose tolerance and prediabetes respectively. Median sensor glucose concentration was 5.0 mmol/L (IQR 4.4–5.4 mmol/L) and 28 children (26.4%) exceeded the hyperglycemic sensor glucose concentration (≥ 7.8 mmol/L), median 1.7% of the total time. In children with a HOMA-IR > 2.5 who reached hyperglycemic excursions during rtCGM, eGFR was strongly correlated with time spent in the hyperglycemic range and indices of glucose variability.

Conclusions. High glucose levels and fluctuations during daily life of children with overweight and obesity with insulin resistance are related with a higher eGFR. These findings support the hypothesis of hyperglycemia-induced hyperfiltration in children with overweight and obesity.

INTRODUCTION

The prevalence of childhood obesity is increasing and parallel to this, the prevalence of diabetes (diabetes in the context of obesity) is increasing as well. The noncommunicable disease risk factor collaboration estimated in 2016 that 124 million children and adolescents worldwide were affected by obesity (1). Various obesity-related comorbidities, such as insulin resistance (IR), are frequently present in children (2). Besides, obesity and its related conditions during childhood are likely to track into adulthood (3) and there is evidence that obesity during childhood leads to chronic kidney disease (CKD) later in life (4). CKD already is a major public health challenge with a global estimated prevalence of about 10% (5), which is likely to increase the following decades resulting from the obesity pandemic. CKD is a major complication of both obesity and diabetes, and diabetes remains one of the leading causes of end-stage kidney disease globally (6).

Traditionally, type 2 diabetes mellitus (T2DM) was considered to be an adult-onset disease resulting from long-term glucose dysregulation and failure of pancreatic β cells to produce insulin. With the childhood obesity pandemic, the prevalence of T2DM in children has increased with 95% over the past 16 years and was estimated to be 0.67 per 1000 children aged 10 to 19 years in 2017 in the United States of America (7). Compared with onset of T2DM in adulthood, adults with onset of T2DM in childhood have a higher incidence of kidney disease. In the TODAY2 study the cumulative incidence of diabetic kidney disease was about 50% mean 13.3 ± 1.8 years after the diagnosis of T2DM (8). This underlines the importance of addressing obesity and glucose dysregulation during childhood.

Whilst T2DM itself is relatively rare in children, precursors of T2DM, such as IR and impaired glucose tolerance (IGT) are more common in children with overweight and obesity. The prevalence of IR is estimated to be up to 72% in children with adiposity, largely depending on methods and definitions used (9). IR and hyperglycemia are important risk factors for obesity- and diabetes related kidney disease (10-12). In a hyperglycemic state the glucose concentration in the ultrafiltrate in the proximal tubule increases, which induces the expression of the sodium-glucose cotransporter 2 (SGLT2) and enhances reabsorption of both sodium and glucose. The enhanced sodium reabsorption causes lower sodium concentrations in the distal tubule which deactivates the tubuloglomerular feedback mechanism, and as a result from dilatation of the afferent glomerular arterioles and increased renin secretion, glomerular filtration rate (GFR) will increase. In this manner, hyperglycemia leads to single nephron hyperfiltration, the hallmark in the early phase of obesity- and diabetes-related kidney disease (10-12).

Also in the absence of T2DM, hyperglycemic glucose excursions are common in children with obesity (13, 14). Though, to the best of our knowledge, no studies exist on the

relation between kidney function and (high) glucose levels in free living conditions in children without diabetes. Therefore, we conducted a cross-sectional study in children with overweight and obesity to examine this correlation in which the glycemic profile was evaluated with both oral glucose tolerance test (OGTT) and using real-time continuous glucose monitoring (rtCGM).

METHODS

Study population, setting and inclusion

Children from this study were enrolled from the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre (MUMC+) MosaKids Children's Hospital. COACH is a lifestyle intervention program for children with overweight and obesity that focusses on a healthier lifestyle, as described previously (15). For this study, only baseline pre-intervention data was used. All 212 children who entered the COACH program between 2011 and 2013 were offered rtCGM. 44 children refused. 58 children were excluded from this study due to missing data. One child with type 1 diabetes and one child with a history of kidney disease was excluded for this study. 2 children were 18 years at the moment of the measurements, and were thus excluded from this study. Finally, 106 children were eligible for inclusion. For this study, the guidelines administered by the Declaration of Helsinki were respected and the Medical Ethical Committee (METC) of the MUMC+ approved this study (METC-number 13-4-130). The study is registered at ClinicalTrial.gov as NCT02091544. Informed consent was obtained from children that were 12 years and older and from all parents or legal guardian.

Clinical examination

Anthropometric data were collected according to relevant guidelines and regulations. Weight was measured using a digital scale (Seca, Chino, CA) and height was measured using a digital stadiometer (De Grood Metaaltechniek, Nijmegen, the Netherlands). BMI and BMI z-score were determined. Overweight, obesity and severe obesity were defined according to the International Obesity Task Force (IOTF) criteria (16). Waist- and hip-circumference were measured and the waist-to-hip ratio was calculated. The equation by Haycock et al. was used to calculate body surface area (BSA) out of weight (kg) and height (cm), $BSA = 0.024265 \times Wt^{0.5378} \times Ht^{0.3964}$ (17).

Analyses of glucose metabolism

The methods for measurements of the glucose metabolism were similar as described previously (13). After obtaining the fasting blood sample an oral glucose tolerance test (OGTT) was performed. 1.75 grams of glucose per kilogram of total bodyweight was dissolved into 200 mL water, with a maximum of 75 grams of glucose in total, and given orally. Plasma glucose concentrations were measured every thirty minutes during

two hours. Impaired fasting glucose (IFG; fasting glucose 5.6–6.9 mmol/L), impaired glucose tolerance (IGT; glucose ≥ 7.8 –< 11.0 mmol/L after 2-hours post-OGTT) and elevated HbA1c concentrations ($\geq 5.7\%$; ≥ 39 mmol/mol) were classified according to the American Diabetes Association (ADA) criteria (18). Prediabetes was defined as IFG or IGT or a HbA1c concentration between 5.7–6.4% (39–46 mmol/mol). Diabetes mellitus was defined as a fasting glucose ≥ 7.0 mmol/L, a 2-hour post-OGTT glucose ≥ 11.1 mmol/L or HbA1c concentration $\geq 6.5\%$ (≥ 48 mmol/mol) (18). Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) in which $HOMA - IR = \frac{\text{fasting glucose } (\frac{mmol}{L}) \times \text{fasting insulin } (\frac{mU}{L})}{22.5}$; a cut-off value of 2.5 was used (19).

In addition to fasting and OGTT data, a continuous glucose monitoring (CGM) sensor (Medtronic) was used to determine glucose concentrations in free-living conditions. The CGM sensor was inserted subcutaneously in the upper leg or arm, depending on the preference of the child. Interstitial fluid glucose levels were measured every five minutes with the CGM sensor for 72 hours. To ensure all sensor blood glucose measurements were obtained in free-living conditions the values of the second and third day (both from 00:00–23:59) were used for analysis (48 hours in total). Calibration of the device required capillary glucose readings three times per day which was obtained through self-monitored capillary glucose samples: fasting, in the afternoon and pre-bedtime using the Accu-Chek (Roche). After 72 hours, the sensor was removed and the data was uploaded to an online software program (Medtronic Carelink-software). Afterwards, 48 hour sensor blood glucose concentrations were calculated. Definitions were used according to the international CGM consensus (20) and the consensus paper from the ADA and European Association for the Study of Diabetes (21). Because the children in this study were unlikely to have glucose concentrations in the hyperglycemic range >10 mmol/L, the cut-off value for hyperglycemia in this study was chosen to be a glucose concentration ≥ 7.8 mmol/L. Since glucose fluctuations are influenced by dietary habits and physical activity during the day, daytime (07.00 am–10.00 pm) and night-time (10.00 pm–7.00 am) sensor glucose concentrations were evaluated separately. The timeframe for day and night was based on the mean self-reported sleeping time. Total sensor glucose AUC were calculated using a standard trapezoidal method. Hypo- and hyperglycemic sensor glucose AUC were also calculated, reflecting the AUC for sensor glucose concentrations < 3.9 mmol/L ≥ 7.8 mmol/L respectively. We used the standard deviation (SD) of sensor glucose and its derived coefficient of variation (CV, which is $CV = \frac{SD \text{ CGM sensor glucose}}{\text{mean CGM sensor glucose}} \times 100$) as indices of glucose variability.

Laboratory analyses

Venous blood samples were collected in fasting state. Fasting plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, triacylglyceride, uric acid and alanine transaminase (ALT) concentrations were determined (Cobas 8000, Roche). Serum insulin and glycated hemoglobin (HbA1c) concentrations were analysed (Immulite-1000,

Siemens Healthcare Diagnostics, and fully automated HPLC Variant II, Bio-Rad Laboratories respectively). Low-density lipoprotein (LDL) cholesterol concentrations were calculated with the equation for Friedewald et al. (22). Urine albumin was measured using an immunoturbidimetric method in a random urine spot. Urine albumin-to-creatinine ratio (UACR) was calculated by dividing the albumin concentration by the creatinine level. Microalbuminuria was defined as an UACR of ≥ 30 mg/g and ≤ 300 mg/g.

Estimated kidney function

Kidney function was estimated using serum creatinine (SCr), which was measured by means of the enzymatic method (Cobas 8000, Roche). SCr was rescaled using reference Q-age and Q-height polynomials respectively (23, 24). Rescaled SCr (SCr/Q) was evaluated using the reference band of [0.67 – 1.33], representing the 2.5th and 97.5th percentile (25). SCr was converted into eGFR using the following equations: full-age spectrum (FAS), both height-independent (FAS-age) as height-dependent (FAS-height) (23, 24); new European Kidney Function Consortium (EKFC) (26); updated bedside Schwartz or CKiD (27); Schwartz-Lyon (28); CKiD under 25 years (CKiDU25) (29); adjusted-creatinine revised Lund-Malmö (LMR18) (30); and CKD-EPI equation with age-adjusted creatinine values (CKD-EPI40) (31).

Blood pressure

Office blood pressure (BP) was measured median 21 times (interquartile range 17-23) with an interval of three minutes between each measurement (Mobil-O-Graph, I.E.M. GmbH, Stolberg, Germany). Mean systolic BP (SBP) and diastolic (DBP) were calculated and turned into percentiles using the pediatric normative tables, based on sex, age and height (32).

Statistical analysis

For the description of the basic features in this study, descriptive statistics were used. Summary statistics are presented as mean $\pm$ standard deviation (normally distributed data) or median (interquartile range, IQR) otherwise. All P-values are two-tailed and results were considered statistically significant when P-values were below 0.05. Statistics were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

ClinicalTrial.gov; Registration Number: NCT02091544.

RESULTS

Characteristics

The characteristics of the 106 children are displayed in Table 5.1. Mean age was 12.37 ± 2.87 years, and 60.4% was female. Mean BMI z-score was 3.42 ± 0.69 and most children (81%) in this cohort had obesity or severe obesity. Males had a significantly higher BMI

z-score compared to females (3.61 vs. 3.29, $p=0.020$), but apart from this, no significant differences were demonstrated between sexes. In Table 5.2, data of SCr/Q and eGFR are presented. Most children had a SCr/Q within the normal reference range, and mean eGFR varied between 101.4 and 121.4 ml/min/1.73m², depending on sex and equation used. No significant differences between the sexes could be demonstrated.

Table 5.1. Characteristics of the children with overweight and obesity, stratified according to sex.

Variable	Total (n=106)	Females (n = 64)	Males (n = 42)	P-value
Age (years)	12.37 ± 2.87	12.34 ± 3.07	12.41 ± 2.58	0.904
BMI z-score	3.42 ± 0.69	3.29 ± 0.67	3.61 ± 0.68	0.020*
Overweight (%)	18.9	21.9	14.3	0.329
Obesity (%)	39.6	37.5	42.9	0.581
Severe obesity (%)	41.5	40.6	42.9	0.820
BSA (m ²)	1.80 (1.55 – 2.09)	1.79 (1.47 – 2.09)	1.80 (1.60 – 2.09)	0.489
Waist-to-hip ratio	0.93 ± 0.08	0.92 ± 0.09	0.93 ± 0.07	0.297
Microalbuminuria (%) ¹	0.9	1.6	0	0.419
Average SBP percentile	88 (78 – 97)	89 (76 – 97)	88 (80 – 96)	0.717
Average DBP percentile	74 (57 – 85)	74 (56 – 86)	74 (57 – 82)	0.657
Total cholesterol (mmol/L)	4.43 ± 0.79	4.52 ± 0.78	4.29 ± 0.81	0.152
LDL-cholesterol (mmol/L)	2.71 ± 0.69	2.79 ± 0.69	2.58 ± 0.68	0.136
HDL-cholesterol (mmol/L)	1.18 ± 0.27	1.17 ± 0.26	1.18 ± 0.29	0.889
Triacylglyceride (mmol/L)	0.99 (0.71 – 1.44)	0.99 (0.71 – 1.52)	1.00 (0.67 – 1.40)	0.829
Uric acid (mg/dL)	4.85 ± 0.79	4.96 ± 0.86	4.78 ± 0.78	0.637
ALT (U/L)	22 (17 – 32)	21 (16 – 29)	23 (17 – 40)	0.237

Data presented as mean ± standard deviation or median (interquartile range) or percentage (%). Abbreviations: BMI, body mass index; BSA, body surface area; SCr/Q-age, serum creatinine normalized using reference Q-age polynomials; SCr/Q-height, serum creatinine normalized using reference Q-height polynomials; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine transaminase. ¹ Data was available for 79 children (48 girls, 31 boys). P-value is obtained from independent samples T-test, Mann-Whitney U test, Pearson's chi-squared test or Fisher's exact test as appropriate. * P-value <0.050.

Glucose metabolism

All included children underwent an OGTT and 48 hour rtCGM, results are displayed in Table 5.3. 60 children (56.6%) had a HOMA-IR > 2.5, suggesting IR. 3.8% and 23.6% of the children had IGT and prediabetes respectively. None of the children had IFG or diabetes. Median sensor glucose concentration was 5.0 mmol/L (IQR 4.4–5.4 mmol/L) and 28 children (26.4%) exceeded the hyperglycemic sensor glucose concentration (≥ 7.8 mmol/L). No significant differences between sexes were demonstrated. In Supplement Table S5.1, the characteristics of children with and without hyperglycemic excursions during rtCGM are displayed. In children with hyperglycemic glucose excursions, median

time in hyperglycemic range was 48 minutes (IQR 15-171 minutes, minimum 5 minutes, maximum 400 minutes). Compared to children who did not exceed a sensor glucose ≥ 7.8 mmol/L, children with hyperglycemic sensor glucose excursions had a significant higher average sensor glucose (5.4 versus 4.8 mmol/L, $p=0.037$).

Table 5.2. Rescaled serum creatinine (SCr/Q) and estimated glomerular filtration rate (eGFR) of the children with overweight and obesity stratified to sex.

Variable	Total (n=106)	Females (n = 64)	Males (n = 42)	P-value
SCr/Q-age	0.93 $\pm$ 0.13	0.93 $\pm$ 0.12	0.94 $\pm$ 0.15	0.588
SCr/Q-age within [0.67 – 1.33] (%)	98.1	98.4	97.6	0.762
SCr/Q-height	0.92 $\pm$ 0.14	0.90 $\pm$ 0.14	0.93 $\pm$ 0.14	0.310
SCr/Q-height within [0.67 – 1.33] (%)	97.2	96.9	97.6	0.821
eGFR (ml/min/1.73m ²)	-	-	-	-
FAS-age	117.3 $\pm$ 16.6	117.8 $\pm$ 16.4	116.5 $\pm$ 17.1	0.691
FAS-height	119.9 $\pm$ 18.7	121.4 $\pm$ 19.1	117.6 $\pm$ 18.0	0.311
EKFC	108.0 $\pm$ 9.1	107.6 $\pm$ 8.2	108.6 $\pm$ 10.3	0.549
CKiD	119.2 $\pm$ 19.6	121.7 $\pm$ 19.7	115.5 $\pm$ 19.1	0.115
Schwartz-Lyon	108.1 $\pm$ 16.9	108.4 $\pm$ 17.5	107.6 $\pm$ 16.0	0.798
CKiDU25	110.7 $\pm$ 15.8	108.7 $\pm$ 15.7	113.9 $\pm$ 15.6	0.098
LMR18	102.5 $\pm$ 10.2	101.4 $\pm$ 9.5	104.0 $\pm$ 11.1	0.192
CKD-EPI40	105.3 $\pm$ 11.4	104.1 $\pm$ 10.8	107.0 $\pm$ 12.2	0.210

Data presented as mean $\pm$ standard deviation or percentage. Abbreviations: SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials; FAS, full-age spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25; LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children. P-value obtained from the independent samples T-test or Pearson's chi-squared test as appropriate.

No other differences between groups were demonstrated, though SCr/Q tends to be lower (and thus eGFR tends to be higher) in children with hyperglycemic sensor glucose excursions.

Correlations between estimated kidney function and glycemic profile

In the total group of children, univariate correlation analysis was performed to examine possible correlations between estimated kidney function and glycemic variables. SCr/Q and some eGFR equations were significantly, though weakly, correlated with daytime rtCGM sensor glucose concentration (correlation coefficients ranging from 0.198-0.248, $p<0.050$) and the area under the curve (AUC) of the total sensor glucose (correlation coefficients ranging from 0.201-0.263, $p<0.050$). No other correlations between estimated kidney function and glycemic variables could be demonstrated.

In Supplement Table S5.2, correlations between time spent in hyperglycemic range estimated kidney function are displayed ($n=28$), stratified according to HOMA-IR. In children with hyperglycemic excursions during rtCGM with a HOMA-IR > 2.5 ($n=15$), time spent in the hyperglycemic range was significantly and strongly correlated with SCr/Q-height (SCr/Q-height, $r = -0.675$, $p = 0.006$; SCr/Q-age, $r = -0.514$, $p = 0.050$) and eGFR (correlation coefficients ranging from 0.554-0.743, $p < 0.05$ (excluding eGFR FAS-age, $r = 0.514$, $p = 0.050$). This correlation was in the opposite direction in children with hyperglycemic excursions during rtCGM with a HOMA-IR ≤ 2.5 ($n=13$); though only significant for eGFR CKiD ($r = -0.631$, $p = 0.021$) and eGFR Schwartz-Lyon ($r = -0.603$, $p = 0.029$). In Figure 5.1, scatterplots including line of best fit and 95% confidence interval error bars are shown for SCr/Q-age (A), SCr/Q-height (B), eGFR FAS-age (C), eGFR FAS-height (D), eGFR CKiD (E) and eGFR CKiDU25 (F) for children with hyperglycemic glucose excursions, stratified according to HOMA-IR ≤ 2.5 ($n=13$, A1-F1) and children with HOMA > 2.5 ($n=15$, A2-F2). After correction for sex, age and BMI z-score, correlations between time spent in hyperglycemic range and estimated kidney function remain present in children with a HOMA-IR > 2.5 ($n=15$). As summarized in Table S5.3, in these multivariable models (with R^2 of about 0.80), age is never a significant independent variable, whereas sex and BMI-z score are in about half of the equations. In the subgroup of children with a HOMA-IR ≤ 2.5 ($n=13$), estimated kidney function and time spent is no longer significant after correction for sex, age and BMI-z score.

Moreover, in children with a HOMA-IR > 2.5 who reached hyperglycemic excursions during 48 hour rtCGM ($n=15$), SCr/Q and eGFR were significantly and strongly correlated with the SD of glucose sensor levels (SCr/Q-age, $r = -0.786$, $p < 0.001$; SCr/Q-height, $r = -0.825$, $p < 0.001$; eGFR correlation coefficients ranging from 0.768-0.854, $p < 0.001$) and CV of glucose sensor levels (SCr/Q-age, $r = -0.582$, $p = 0.023$; SCr/Q-height, $r = -0.568$, $p = 0.027$; eGFR correlation, coefficients ranging from 0.539-0.629, $p < 0.050$). In children with hyperglycemic excursions with a HOMA-IR ≤ 2.5 ($n=13$), no significant correlations between SCr/Q or eGFR and SD or CV of glucose sensor levels were demonstrated.

Table 5.3. Variables of glucose metabolism determined in fasting state and during OGTT and rtCGM.

Variable	Total (n=106)	Females (n = 64)	Males (n = 42)	P-value
Fasting plasma glucose (mmol/L)	4.1 ± 0.5	4.1 ± 0.6	4.1 ± 0.5	0.799
Fasting plasma insulin (mU/L)	15.5 (10.5 – 23.6)	17.0 (11.0 – 23.8)	12.7 (8.5 – 23.0)	0.146
HOMA-IR	2.86 (1.87 – 4.15)	3.16 (2.07 – 4.17)	2.36 (1.61 – 4.16)	0.155
HOMA-IR > 2.5 (%)	56.6	64.1	45.2	0.056
HbA1c (%)	5.4 (5.1 – 5.6)	5.4 (5.2 – 5.7)	5.3 (5.1 – 5.5)	0.137
HbA1c ≥ 5.7% (%)	21.7	25.0	16.7	0.370
Plasma glucose during OGTT (mmol/L)	-	-	-	-
30 minutes	6.7 (5.7 – 7.9)	6.6 (5.7 – 8.2)	6.8 (5.9 – 7.5)	0.713
60 minutes	6.3 (5.2 – 7.5)	6.3 (5.3 – 7.5)	6.1 (5.1 – 7.5)	0.696
90 minutes	5.8 (4.8 – 6.8)	6.0 (5.0 – 6.9)	5.4 (4.6 – 6.6)	0.131
120 minutes	5.4 (4.7 – 6.1)	5.5 (4.9 – 5.9)	5.3 (4.5 – 6.3)	0.584
Plasma insulin during OGTT (mU/L)	-	-	-	-
30 minutes	114.5 (72.1 – 212.0)	114.5 (76.3 – 220.3)	115.0 (62.2 – 204.8)	0.518
60 minutes	82.1 (45.8 – 167.5)	91.0 (60.4 – 172.0)	73.7 (41.8 – 111.0)	0.083
90 minutes	73.9 (41.9 – 148.3)	87.7 (45.5 – 168.5)	61.3 (38.8 – 120.1)	0.131
120 minutes	70.3 (43.9 – 132.0)	76.7 (45.1 – 133.0)	59.7 (41.7 – 103.0)	0.321
IGT (%)	3.8	6.3	0.0	0.108
Prediabetes (%)	23.6	28.1	16.7	0.225
Average rtCGM sensor glucose (mmol/L)	5.0 (4.4 – 5.4)	5.0 (4.4 – 5.6)	4.9 (4.3 – 5.4)	0.310
Day	5.2 (4.8 – 5.5)	5.3 (4.9 – 5.5)	5.2 (4.8 – 5.6)	0.833
Night	5.0 (4.4 – 5.4)	5.0 (4.4 – 5.6)	4.9 (4.3 – 5.4)	0.310
SD (mmol/L)	0.7 (0.6 – 0.9)	0.7 (0.6 – 0.9)	0.7 (0.6 – 0.9)	0.329
CV (%)	13.7 (11.0 – 18.1)	14.4 (10.9 – 19.3)	13.6 (11.7 – 16.9)	0.574
Peak rtCGM sensor glucose (mmol/L)	7.0 (6.3 – 7.8)	7.2 (6.3 – 7.8)	6.9 (6.4 – 7.8)	0.698
Day	7.0 (6.3 – 7.7)	7.0 (6.2 – 7.8)	6.9 (6.3 – 7.5)	0.808
Night	6.1 (5.7 – 6.6)	6.1 (5.7 – 6.7)	6.1 (5.6 – 6.6)	0.719
Minimum rtCGM sensor glucose (mmol/L)	3.4 (3.1 – 3.9)	3.4 (3.1 – 3.9)	3.6 (3.1 – 3.9)	0.340
Day	3.8 (3.4 – 4.2)	3.8 (3.4 – 4.2)	3.9 (3.4 – 4.2)	0.466
Night	3.6 (3.1 – 4.0)	3.4 (3.1 – 3.9)	3.6 (3.1 – 4.1)	0.311
AUC rtCGM (mmol/L)	-	-	-	-
Total glucose	14528.0 (13618.0 – 15272.8)	14560.1 (13564.9 – 15163.5)	14480.9 (13634.7 – 15582.9)	0.670
Glucose < 3.9 mmol/L (mmol/L) ¹	841.0 (354.0 – 1556.5)	901.5 (434.5 – 1548.0)	809.0 (269.5 – 1738.5)	0.916

Table 5.3. Variables of glucose metabolism determined in fasting state and during OGTT and rtCGM.
(continued)

Variable	Total (n=106)	Females (n = 64)	Males (n = 42)	P-value
Glucose $\geq$ 7.8 mmol/L (mmol/L) ²	478.3 (157.0 – 1590.5)	478.3 (155.5 – 1590.5)	683.6 (167.3 – 1519.6)	0.875
Time rtCGM (minutes)	-	-	-	-
Glucose < 3.9 mmol/L	120.0 (0.0 – 365.0)	142.5 (3.8 – 372.5)	75.0 (0.0 – 353.8)	0.483
Glucose between 3.9 and 7.8 mmol/L	2712.5 (2495.0 – 2861.3)	2702.5 (2495.0 – 2862.5)	2745.0 (2506.3 – 2865.0)	0.504
Glucose $\geq$ 7.8 mmol/L	0.0 (0.0 – 7.5)	0.0 (0.0 – 12.5)	0.0 (0.0 – 3.8)	0.583

Data presented as mean $\pm$ standard deviation (SD), median (interquartile range) or percentage (%).

¹ Total n=77 (48 females, 29 males), ² Total n=28 (18 females, 10 males). Abbreviations: HOMA-IR, homeostasis model assessment for insulin resistance; HbA1c, glycated hemoglobin; OGTT, oral glucose tolerance test; IGT, impaired glucose tolerance; rtCGM, real-time continuous glucose monitoring; CV, coefficient of variation; AUC, area under the curve. P-value is obtained from independent samples T-test, Mann-Whitney U test, Pearson's chi-squared test or Fisher's exact test as appropriate. * P-value <0.050.

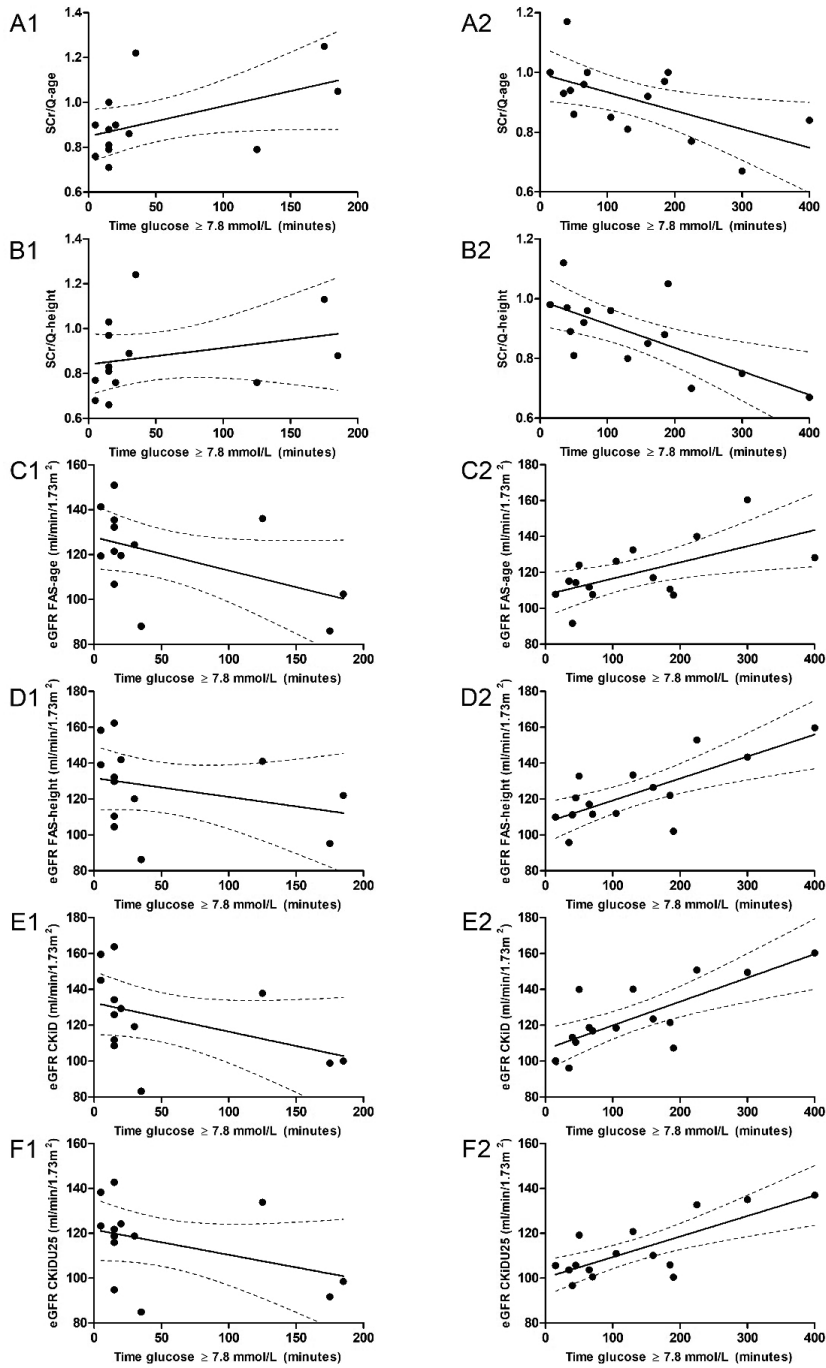


Figure 5.1. Scatterplots for SCr/Q-age (A), SCr/Q-height (B), eGFR FAS-age (C), FAS-height (D), CKiD (E) and CKiDU25 (F) against time in hyperglycemic state (minutes) in children with hyperglycemic sensor glucose excursions (glucose ≥ 7.8 mmol/L). On the left side, children with a HOMA-IR ≤ 2.5 are shown ($n=13$; A1-F1) and on the right side children with a HOMA-IR > 2.5 ($n=15$; A2-F2). Included are linear regression lines and 95% confidence interval error bars.

DISCUSSION

In this study we examined the glycemic profile in free-living conditions of 106 children with overweight and obesity without diabetes and without overt kidney disease, and correlated these findings with estimated kidney function. The main finding of this study is that, in children with overweight and obesity with IR and hyperglycemic glucose excursions, time spent in hyperglycemic range in free living conditions is strongly correlated with eGFR. Most children had an estimated kidney function within the normal reference range, which was shown previously in a comparable (though much bigger) cohort (33, 34). Moreover, in the studied children, different stages of glucose dysregulation were already highly present: 56.6% of the children had a HOMA-IR > 2.5 (suggesting IR), and 3.8% and 23.6% of the children had IGT and prediabetes respectively. During 48 hour rtCGM, hyperglycemic sensor glucose excursions were present in 26.4% of the children with overweight and obesity, and median time in hyperglycemic range was 48 minutes (IQR 15-171 minutes), which was 1.7% of total time. Compared to children without sensor glucose values ≥ 7.8 mmol/L, children with hyperglycemic glucose excursions had a significant higher average sensor glucose during rtCGM; remarkably no other anthropometric or metabolic variables did significantly differ between the groups.

The childhood obesity pandemic will lead to a major public health problem, not in the least for future kidney health. Parallel to this, the prevalence of diabetes is dramatically increasing as well, which will compromise future kidney health even more since CKD is a major complication of both obesity and diabetes. As described, the hallmark of the early phase of obesity- and diabetes-related CKD is hyperglycemia/SGLT2-induced single nephron hyperfiltration, a state of supraphysiological GFR (10-12). Glomerular hyperfiltration is associated with loss of kidney function (35) and is associated with all-cause mortality and adverse cardiovascular outcomes (36, 37). Glomerular hyperfiltration can be 'absolute' or 'relative', in case of respectively, a normal or reduced number of functioning nephrons. Independent of 'absolute' or 'relative' hyperfiltration, persistent increase in the single-nephron GFR eventually leads to hyperfiltration injury via the mechanism that was already hypothesized by Brenner et al. in 1996 (38): glomerular hyperfiltration is associated with glomerular hypertension that leads, via podocyte detachment, to proteinuria, glomerulosclerosis and eventually decline in kidney function (39). In clinical setting, a high GFR (so whole-kidney GFR, the sum of the single-nephron GFR) is often used as a surrogate marker for single-nephron glomerular hyperfiltration. It is important to consider that this surrogate marker is not perfect but inevitable, since determining single-nephron GFR and nephron mass in routine clinical setting is still impossible (40).

In this study, the glycemic profile was evaluated both in clinical setting (using OGTT) as well as in real-time (using rtCGM). RtCGM provides serial interstitial glucose

measurements during daily life and, while the technique has its origin in patients with (type 1) diabetes, it is increasingly used to study glycemic profile (like glycemic excursions and variability) in patients with risk factors for glucose dysregulation, like in case of obesity. While most studies evaluating rtCGM in obesity have been conducted in adults, there are studies conducted in children as well (14). In a scoping review published in 2021, 8 rtCGM studies in children with obesity are described: cohort size ranges from 17 to 118 children and range of rtCGM wear was in between 1 and 8 days (14). In the largest cohort study described in the review, Chan et al. published on rtCGM data in 118 children with overweight and obesity across a spectrum of glycemic states from normal to diabetes (median age 14.1 years, 35% male, median BMI z-score 2.3) and related these findings to HbA1c levels among others (41). In the subgroup of children (n=51) with a median HbA1c <5.7% (which is similar to our cohort), average sensor glucose was median 108 mg/dL (= 6.0 mmol/L) (and thus higher than the glucose levels in our cohort (median 5.0 mmol/L)). Time spent in hyperglycemia (≥ 140 mg/dL = 7.8 mmol/L) was median 1.2% of total time (range 0.0-77.3) for children with HbA1c levels <5.7% (in our cohort: 48 minutes during 48 hours = 1.7%). None of the studies described in the scoping review included (estimated) kidney function. More recently, Chylińska-Frątczak et al. published on 143 children with adiposity (mean age 13.4 ± 2.5 years, 54.5% females, mean BMI z-score 2.2 ± 0.4) of whom 46 underwent rtCGM (42). Average sensor glucose was median 101.2 mg/dL (= 5.6 mmol/L) and time spent in hyperglycemia (≥ 140 mg/dL = 7.8 mmol/L) was median 1.4% of total time.

To the best of our knowledge, this study is the first to examine correlations between estimated kidney function and elevated glucose levels in free living conditions in children with overweight and obesity without diabetes. In (young) adults, multiple studies demonstrated the correlation between pre-diabetes glucose dysregulation and estimated (or measured) GFR (and or presence of glomerular hyperfiltration) (43-48).

Finally, not only the hyperglycemic state, but also the glycemic variability is thought to be a contributing factor in the development of CKD with or without diabetes (49). In this study, in children with a HOMA-IR > 2.5 and hyperglycemic excursions during 48 hour rtCGM, SCr/Q and eGFR were strongly and significantly correlated with the standard deviation (SD) of glucose sensor levels and coefficient of variation (CV) of glucose sensor levels.

Strengths of this study include the relatively large sample size of rtCGM in children with overweight and obesity without diabetes. Limitations are the low incidence of IGT, which is inherent to the pediatric population, the fact that GFR was estimated instead of measured, and that GFR was estimated with only SCr, whereas other endogenic markers (such as cystatin C) are of interest as well.

In conclusion, high glucose levels and fluctuations during daily life of children with overweight and obesity with insulin resistance are related with a higher eGFR. These findings support the hypothesis of hyperglycemia-induced hyperfiltration in children with overweight and obesity. Prospective studies are needed to further evaluate the correlation between glycemic profile and estimated kidney function in children with overweight and obesity.

REFERENCES

1. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet*. 2017;390(10113):2627-42
2. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic Risks and Severity of Obesity in Children and Young Adults. *N Engl J Med*. 2015 Oct;373(14):1307-17
3. Juhola J, Magnussen CG, Viikari JS, Kähönen M, Hutri-Kähönen N, Jula A, et al. Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. *J Pediatr*. 2011;159(4):584-90
4. Jadresic L, Silverwood RJ, Kinra S, Nitsch D. Can childhood obesity influence later chronic kidney disease? *Pediatr Nephrol*. 2019;34(12):2457-77
5. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. *Lancet*. 2017;389(10075):1238-52
6. Farag YM, Gaballa MR. Diabesity: an overview of a rising epidemic. *Nephrol Dial Transplant*. 2011;26(1):28-35
7. Lawrence JM, Divers J, Isom S, Saydah S, Imperatore G, Pihoker C, et al. Trends in Prevalence of Type 1 and Type 2 Diabetes in Children and Adolescents in the US, 2001-2017. *JAMA*. 2021 Aug 24;326(8):717-727
8. Bjornstad P, Drews KL, Caprio S, Gubitosi-Klug R, Nathan DM, Tesfaldet B, et al. Long-Term Complications in Youth-Onset Type 2 Diabetes. *N Engl J Med*. 2021;385(5):416-26
9. van der Aa MP, Knibbe CA, Boer A, van der Vorst MM. Definition of insulin resistance affects prevalence rate in pediatric patients: a systematic review and call for consensus. *J Pediatr Endocrinol Metab*. 2017;30(2):123-31
10. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *J Am Soc Nephrol*. 2017;28(4):1023-39
11. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol*. 2016;12(8):453-71
12. Sawyer A, Zeitler E, Trachtman H, Bjornstad P. Kidney Considerations in Pediatric Obesity. *Curr Obes Rep*. 2023;12(3):332-44
13. Rijks J, Karnebeek K, van Dijk JW, Dorenbos E, Gerver WJ, Stouthart P, et al. Glycaemic Profiles of Children With Overweight and Obesity in Free-living Conditions in Association With Cardiometabolic Risk. *Sci Rep*. 2016;6:31892
14. Hegedus E, Salvy SJ, Wee CP, Naguib M, Raymond JK, Fox DS, et al. Use of continuous glucose monitoring in obesity research: A scoping review. *Obes Res Clin Pract*. 2021;15(5):431-8
15. van Dam M, Rijks J, Dorenbos E, Horuz F, van Dael K, Vreugdenhil A. The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity. *Sci Rep*. 2019;9(1):4504
16. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes*. 2012;7(4):284-94
17. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr*. 1978;93(1):62-6
18. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care*. 2018;41(Suppl 1):S13-s27

19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-9
20. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. *Diabetes Care*. 2017;40(12):1631-40
21. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range. *Diabetes Care*. 2019;42(8):1593-603
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499-502
23. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol*. 2012;27(6):973-9
24. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aïssa A, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant*. 2014;29(5):1082-91
25. Pottel H, Hoste L, Delanaye P. Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m². *Pediatr Nephrol*. 2015;30(5):821-8
26. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, et al. Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate: A Cross-sectional Analysis of Pooled Data. *Ann Intern Med*. 2021;174(2):183-91
27. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20(3):629-37
28. De Souza VC, Rabilloud M, Cochat P, Selistre L, Hadj-Aïssa A, Kassai B, et al. Schwartz formula: is one k-coefficient adequate for all children? *PloS One*. 2012;7(12):e53439
29. Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ. Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. *Kidney Int*. 2021;99(4):948-56
30. Björk J, Nyman U, Delanaye P, Grubb A, Larsson A, Vranken L, et al. A novel method for creatinine adjustment makes the revised Lund-Malmö GFR estimating equation applicable in children. *Scand J Clin Lab Invest*. 2020;80(6):456-63
31. Björk J, Nyman U, Larsson A, Delanaye P, Pottel H. Estimation of the glomerular filtration rate in children and young adults by means of the CKD-EPI equation with age-adjusted creatinine values. *Kidney Int*. 2021;99(4):940-7
32. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics*. 2017;140(3)
33. van Dam M, Pottel H, Vreugdenhil ACE. Creatinine-based GFR-estimating equations in children with overweight and obesity. *Pediatr Nephrol*. 2022;37(10):2393-403
34. van Dam M, Pottel H, Vreugdenhil ACE. Relation between obesity-related comorbidities and kidney function estimation in children. *Pediatr Nephrol*. 2023;38(6):1867-76
35. Cachat F, Combescure C, Cauderay M, Girardin E, Chehade H. A systematic review of glomerular hyperfiltration assessment and definition in the medical literature. *Clin J Am Soc Nephrol*. 2015 Mar 6;10(3):382-9
36. Park M, Yoon E, Lim YH, Kim H, Choi J, Yoon HJ. Renal hyperfiltration as a novel marker of all-cause mortality. *J Am Soc Nephrol*. 2015;26(6):1426-33

37. Kanbay M, Ertuglu LA, Afsar B, Ozdogan E, Kucuksumer ZS, Ortiz A, et al. Renal hyperfiltration defined by high estimated glomerular filtration rate: A risk factor for cardiovascular disease and mortality. *Diabetes Obes Metab.* 2019 Nov;21(11):2368-2383
38. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int.* 1996;49(6):1774-7
39. Cortinovis M, Perico N, Ruggenenti P, Remuzzi A, Remuzzi G. Glomerular hyperfiltration. *Nat Rev Nephrol.* 2022;18(7):435-51
40. Denic A, Glassock RJ, Rule AD. Single-Nephron Glomerular Filtration Rate in Healthy Adults. *N Engl J Med.* 2017;377(12):1203-4
41. Chan CL, Pyle L, Newnes L, Nadeau KJ, Zeitler PS, Kelsey MM. Continuous glucose monitoring and its relationship to hemoglobin A1c and oral glucose tolerance testing in obese and prediabetic youth. *J Clin Endocrinol Metab.* 2015;100(3):902-10
42. Chylińska-Frątczak A, Michalak A, Baranowska-Jaźwiecka A, Mianowska B, Szadkowska A. Incidence of hyperglycaemic disorders in children and adolescents with obesity. *Pediatr Endocrinol Diabetes Metab.* 2022;28(4):274-80
43. Tomaszewski M, Charchar FJ, Maric C, McClure J, Crawford L, Grzeszczak W, et al. Glomerular hyperfiltration: a new marker of metabolic risk. *Kidney Int.* 2007;71(8):816-21
44. Puijm M, Wuerzner G, Maillard M, Bovet P, Renaud C, Bochud M, et al. Glomerular hyperfiltration and increased proximal sodium reabsorption in subjects with type 2 diabetes or impaired fasting glucose in a population of the African region. *Nephrol Dial Transplant.* 2010;25(7):2225-31
45. Melsom T, Mathisen UD, Ingebretsen OC, Jenssen TG, Njolstad I, Solbu MD, et al. Impaired fasting glucose is associated with renal hyperfiltration in the general population. *Diabetes Care.* 2011;34(7):1546-51
46. Okada R, Yasuda Y, Tsushita K, Wakai K, Hamajima N, Matsuo S. Glomerular hyperfiltration in prediabetes and prehypertension. *Nephrol Dial Transplant.* 2012;27(5):1821-5
47. Sun ZJ, Yang YC, Wu JS, Wang MC, Chang CJ, Lu FH. Increased risk of glomerular hyperfiltration in subjects with impaired glucose tolerance and newly diagnosed diabetes. *Nephrol Dial Transplant.* 2016;31(8):1295-301
48. Melsom T, Schei J, Stefansson VT, Solbu MD, Jenssen TG, Mathisen UD, et al. Prediabetes and Risk of Glomerular Hyperfiltration and Albuminuria in the General Nondiabetic Population: A Prospective Cohort Study. *Am J Kidney Dis.* 2016 Jun;67(6):841-50
49. Subramanian S, Hirsch IB. Diabetic Kidney Disease: Is There a Role for Glycemic Variability? *Curr Diab Rep.* 2018 Feb 15;18(3):13.

SUPPLEMENTARY INFORMATION

Supplementary Information Table S5.1. Characteristics of the children with overweight and obesity, stratified according to the presence of hyperglycemic sensor glucose excursions during rtCGM (sensor glucose ≥ 7.8 mmol/L).

Variable	No hyperglycemic sensor glucose excursions during 48 hour rtCGM (n=78)	Hyperglycemic sensor glucose excursions (≥ 1) during 48 hour rtCGM (n=28) ¹	P-value
Age (years)	12.17 (10.29 – 15.33)	11.92 (10.34 – 13.70)	0.554
Female (%)	59.0	64.3	0.622
BMI z-score	3.43 (3.04 – 3.86)	3.28 (2.80 – 4.09)	0.680
Waist-to-hip ratio	0.94 (0.87 – 0.99)	0.90 (0.84 – 0.96)	0.166
Average SBP percentile	89 (80 – 96)	88 (69 – 97)	0.962
Average DBP percentile	73 (53 – 82)	76 (63 – 87)	0.137
Total cholesterol (mmol/L)	4.4 (4.0 – 4.9)	4.4 (3.9 – 5.0)	0.883
LDL-cholesterol (mmol/L)	2.8 (2.3 – 3.1)	2.4 (2.1 – 3.3)	0.430
HDL-cholesterol (mmol/L)	1.1 (0.9 – 1.4)	1.2 (1.0 – 1.4)	0.307
Triacylglyceride (mmol/L)	0.93 (0.62 – 1.39)	1.09 (0.94 – 1.58)	0.072
ALT (U/L)	21 (17 – 31)	24 (15 – 40)	0.503
SCr/Q-age	0.93 (0.86 – 1.03)	0.90 (0.81 – 1.00)	0.237
SCr/Q-height	0.92 (0.81 – 1.04)	0.88 (0.76 – 0.97)	0.137
eGFR	-	-	-
FAS-age	114.8 (103.9 – 125.0)	119.5 (107.7 – 132.4)	0.237
FAS-height	116.3 (103.5 – 133.1)	122.0 (110.6 – 140.6)	0.137
EKFC	110.0 (102.2 – 113.7)	111.4 (107.9 – 114.9)	0.258
CKiD	117.3 (103.5 – 133.1)	120.3 (109.1 – 140.1)	0.118
Schwartz-Lyon	105.5 (92.6 – 118.8)	111.0 (98.9 – 124.8)	0.076
CKiDU25	106.7 (97.4 – 121.0)	113.5 (101.3 – 124.0)	0.190
LMR18	101.6 (94.3 – 109.1)	104.9 (98.7 – 111.6)	0.274
CKD-EPI40	108.5 (96.0 – 112.8)	110.5 (103.5 – 114.5)	0.280
HOMA-IR	2.76 (1.87 – 4.10)	2.87 (1.85 – 4.93)	0.514
HOMA-IR > 2.5 (%)	57.7	53.6	0.706
HbA1c (%)	5.4 (5.1 – 5.6)	5.4 (5.2 – 5.6)	0.737
HbA1c $\geq 5.7\%$ (%)	21.8	21.4	0.918
IGT (%)	2.6	7.1	0.285
Prediabetes (%)	23.1	25.0	0.944
Average rtCGM sensor glucose (mmol/L)	4.8 (4.4 – 5.4)	5.4 (4.4 – 5.7)	0.034*

Data presented as median (interquartile range) or percentage. ¹ Median time in hyperglycemic range (sensor glucose ≥ 7.8 mmol/L) was 48 minutes (IQR 15–171 minutes, minimum 5 minutes, maximum 400 minutes). Abbreviations: rtCGM, real-time continuous glucose monitoring; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high

density lipoprotein; ALT, alanine transaminase; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials; FAS, full-age spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25; LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children. HOMA-IR, homeostasis model assessment for insulin resistance; HbA1c, glycated hemoglobin; IGT, impaired glucose tolerance. P-value is obtained from Mann-Whitney U test or Pearson's chi-squared test or Fisher's exact test as appropriate. * P-value <0.050.

Supplementary Information Table S5.2. Spearman correlations between time spent in hyperglycemic range (glucose ≥ 7.8 mmol/L) during rtCGM and SCr, SCr/Q and SCr-based GFR-estimating equations, stratified according to HOMA-IR ≤ 2.5 or HOMA > 2.5 .

	Time spent in hyperglycemic range (glucose ≥ 7.8 mmol/L) in children with HOMA-IR ≤ 2.5 (n=13)	P-value	Time spent in hyperglycemic range (glucose ≥ 7.8 mmol/L) in children with HOMA-IR > 2.5 (n=15)	P-value
SCr (mg/dL)	0.680	0.011*	-0.696	0.004*
SCr/Q-age	0.478	0.098	-0.514	0.050
SCr/Q-height	0.427	0.145	-0.675	0.006*
eGFR (ml/min/1.73m ²)	-	-	-	-
FAS-age	-0.478	0.098	0.514	0.050
FAS-height	-0.427	0.145	0.675	0.006*
EKFC	-0.461	0.113	0.632	0.011*
CKiD	-0.631	0.021*	0.743	0.002*
Schwartz-Lyon	-0.603	0.029*	0.657	0.008*
CKiDU25	-0.456	0.118	0.611	0.016*
LMR18	-0.385	0.194	0.554	0.032*
CKD-EPI40	-0.385	0.194	0.582	0.023*

Data presented as Spearman's correlation coefficient. Abbreviations: rtCGM, real-time continuous glucose monitoring; HOMA-IR, homeostasis model assessment for insulin resistance; SCr, serum creatinine; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials; FAS, full-age spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25; LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children. * P-value <0.05.

Supplementary Information Table S5.3. Summary of multivariable models with estimated kidney function as dependent variables and age, sex, BMI z-score and time spent in hyperglycemic range as independent variables, stratified according to HOMA-IR ≤ 2.5 or HOMA > 2.5 .

Dependent variable	HOMA-IR ≤ 2.5 (n=13)				HOMA-IR > 2.5 (n=15)			
	Independent variables (p-values)				Independent variables (p-values)			
	Age	Sex	BMI z-score	Time spent in hyperglycemic range	Age	Sex	BMI z-score	Time spent in hyperglycemic range
SCr/Q-age	NS	NS	NS	NS	NS	NS	0.0014	0.0012
SCr/Q-height	NS	NS	NS	NS	NS	0.0356	NS	0.0041
eGFR (ml/min/1.73m ²)								
FAS-age	NS	NS	NS	NS	NS	NS	0.0011	0.0008
FAS-height	NS	NS	NS	NS	NS	NS	NS	0.0021
EKFC	NS	NS	NS	NS	NS	0.0443	0.0036	0.0028
CKiD	NS	NS	NS	NS	NS	NS	0.0255	0.0003
Schwartz-Lyon	NS	NS	NS	NS	NS	NS	NS	0.0021
CKiDU25	NS	NS	NS	NS	NS	NS	NS	0.0013
LMR18	NS	NS	NS	NS	NS	0.0145	0.0007	0.0005
CKD-EPI40	NS	NS	NS	NS	NS	0.0133	0.0018	0.0014

Data presented as not significant (NS) or p values. Abbreviations: HOMA-IR, homeostasis model assessment for insulin resistance; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials; FAS, full-age spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25; LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children.

6

The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity

Mark van Dam, Jesse Rijks, Elke Dorenbos, Flore Horuz, Karin van Dael, Anita Vreugdenhil

Sci Rep. 2019 Mar 14;9(1):4504

ABSTRACT

Obesity causes modifications in the kidneys reversed by weight loss in adults. There is little data on renal function and effects of weight loss in children with obesity. The aim of this prospective study was to examine renal function and effect of a lifestyle intervention in children with overweight, obesity and morbid obesity. Two hundred forty-five children (age 12.4 ± 3.3 years, 40% boys, BMI z-score 3.46 ± 0.70) participating in an out-patient lifestyle intervention were included. Children with at least 12 months follow-up ($n = 144$ (58.8%)) were included in the longitudinal study. Anthropometry, blood analysis and blood pressure measurements were performed at baseline and follow-up.

Glomerular filtration rate (GFR) was estimated using the Schwartz and FAS equation. eGFR was de-indexed using body surface area. Different cut-off points for defining glomerular hyperfiltration were used for stratification. Depending on the definition and equation used, glomerular hyperfiltration was present in 2% to 18% of the participants. After intervention, de-indexed eGFR decreased significantly in children with baseline glomerular hyperfiltration, depending on the eGFR equation and definition for glomerular hyperfiltration used. No associations of changes in eGFR with changes in BMI z-score, blood pressure or parameters of glucose and lipid metabolism were found.

In conclusion, after one year of lifestyle intervention, eGFR decreases in hyperfiltrating children and adolescents with overweight, obesity and morbid obesity. eGFR and changes over time in children with obesity depend on eGFR equation used and on de-indexing for body surface area.

INTRODUCTION

Obesity is known to be an independent risk factor for the development of chronic kidney disease (1, 2). As a consequence of the increasing prevalence of childhood obesity, the prevalence of obesity-related glomerulopathy (ORG) is likely to increase. Obesity causes structural, haemodynamic and metabolic modifications in the kidneys, that are probably the result of (failed) compensatory mechanisms, leading to ORG (3, 4). ORG is histologically defined by glomerular hypertrophy and focal segmental glomerulosclerosis, that can already be present in children and adolescents with obesity (2, 5). A hallmark of the early phase of ORG is an increase in whole-kidney glomerular filtration rate (GFR). Albeit the pathogenesis of this glomerular hyperfiltration is not completely understood, it is known that glomerular hyperfiltration eventually results in podocyte detachment, proteinuria and progression to chronic kidney disease (2). Moreover, glomerular hyperfiltration has been found to be an independent risk factor for all-cause mortality in an apparently healthy population (6).

In adults with obesity and chronic kidney disease weight loss improves proteinuria, albuminuria and normalizes the GFR (7). Despite extensive research on the effects of weight loss on renal function in adults with obesity, to the best of our knowledge, no research has investigated these effects in children. Therefore, we conducted a prospective study to assess the effect of one year of interdisciplinary lifestyle intervention on renal function in children with overweight, obesity and morbid obesity and the intermediary role of anthropometric changes and cardiovascular risk markers.

METHODS

Setting and study inclusion

Children and adolescents with overweight, obesity and morbid obesity were recruited from December 2010 to April 2014 from the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre. Within COACH, the health status of children with overweight and (morbid) obesity was evaluated, and children were treated and monitored as described previously (8). Briefly, participation in the lifestyle intervention of COACH started with a comprehensive assessment aimed to exclude underlying syndromic or endocrine conditions of overweight, to evaluate complications and risk factors, and to gain insight in behaviour and (family) functioning. After the assessment, all children and their families were offered on-going, tailored and individual guidance with focus on lifestyle changes on a frequent basis at the outpatient clinic. Furthermore, participation in sports activities in groups and activities aimed at increasing nutritional knowledge were offered. A follow-up assessment including all the examinations performed during the initial assessment was offered annually

to all children. Exclusion criteria for this study were secondary causes of overweight, pre-existent renal disease, current use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers, psychomotor retardation, diabetes mellitus, cardiac disease and/or malignancies. Due to the continuous inflow of children in the COACH program, the moment of inclusion differed for each participant. The study was conducted according to the guidelines administered by the Declaration of Helsinki and approved by the medical ethical committee of the MUMC+ . Informed consent was obtained from both participants and parents or legal guardian.

Clinical assessment and anthropometry

All methods were carried out in accordance with relevant guidelines and regulations. A detailed history was taken from both participants and parents. In addition, all participants were subjected to a physical examination. Anthropometric data were collected while children were barefoot and wearing only underwear. Weight was determined using a digital scale (Seca, Chino, CA) and length was measured using a digital stadiometer (De Grood Metaaltechniek, Nijmegen, the Netherlands). Overweight, obesity and morbid obesity were defined according to the International Obesity Task Force criteria (9). Waist circumference was measured with a non-elastic measuring tape at the end of a natural breath at midpoint between the top of the iliac crest and the lower margin of the last palpable rib. Hip circumference was measured with a non-elastic measuring tape at the maximum circumference over the bottom. Waist and hip circumference z-score was determined according the Dutch reference values for children (10). Puberty stage was determined by the physicians using the Tanner G/M stadia (11, 12). When no Tanner stadium was reported, girls > 14.3 years and boys > 15.0 years were classified as Tanner G/M stage 4. Tanner stages were subsequently divided in three subgroups: prepubertal (Tanner G/M stage 1), peripubertal (Tanner G/M stage 2-3) and postpubertal (Tanner G/M stage 4-5) (13).

Metabolic measurements and analyses

Venous blood samples were collected after a minimum of 8 hours overnight fasting. Fasting plasma glucose concentrations and serum total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triacylglyceride concentrations were determined with the Cobas 8000 modular analyser (Roche). Serum insulin concentrations were analysed with the Immulite-1000 (Siemens Healthcare Diagnostics). Glycated haemoglobin (HbA1c) concentrations were determined with the fully automated HPLC Variant II (Bio-Rad Laboratories). After obtaining the fasting blood sample an oral glucose tolerance test (OGTT) was performed. 1.75 grams of glucose per kilogram of bodyweight was dissolved into 200 mL water, with a maximum of 75 grams of glucose in total, and given orally. Plasma blood glucose concentrations were measured every thirty minutes during two hours. Creatinine was measured in serum by means of spectrometry (Cobas 8000 modular analyser series, Roche Diagnostics USA).

GFR estimation and defining glomerular hyperfiltration

Glomerular filtration rate (GFR) was estimated by means of the equation by Schwartz et al. (14): estimated GFR (eGFR; ml/min/1.73m²) = 0.413 x height (cm) / serum creatinine (mg/dL)). Moreover, since serum creatinine in children depends on age and gender, and the aim of this study was to evaluate serum creatinine independent of age and gender, serum creatinine of individual subjects was normalized with the median serum creatinine value at the corresponding age and gender. So, we also estimated GFR using the Full Age Spectrum (FAS) equation, proposed and validated by Pottel et al.: eGFR = 107.3 / (serum creatinine (mg/dL) / Q), in which Q is the median value of the serum creatinine defined according gender and age (15, 16). Since all current eGFR equations have been designed for GFR indexed with body surface area (BSA) (15), eGFR was de-indexed using the BSA. BSA was estimated using the Haycock equation: BSA = 0.02465 x Wt^{0.5378} x Ht^{0.3964} (with Wt as weight in kilograms and Ht as height in centimetres) (17, 18). De-indexed eGFR was then calculated by multiplying eGFR values by BSA and dividing by 1.73 m².

For defining glomerular hyperfiltration, several cut-off values are used. (19) Therefore, in this study, different cut-off values were used for stratification: an eGFR of >135ml/min/1.73m², according to the 90th percentile of a cohort described by Piepsz et al. (20), an eGFR >140ml/min/1.73m² (21) and an eGFR >150ml/min/1.73m² (22), based on reference values of Pottel et al. (23): a median GFR of 107.3 mL/min/1.73m² with a standard deviation of 21.5 mL/min/1.73m² leads to a 97.5th percentile of 150 mL/min/1.73m². The amount of microalbuminuria was estimated by means of a random urine spot (RUS). Albumin was measured in a quantitative manner using a immunoturbidimetric method and the urine albumin-to-creatinine ratio (UACR) was calculated by dividing the albumin concentration by the creatinine level. Exact determination of the UACR was not possible <1.77mg/g. Microalbuminuria was defined as an UACR of ≥30mg/g and ≤300mg/g. (24)

Blood pressure

Daytime blood pressure was measured approximately 20 times with an interval of three minutes between each measurement using the Mobil-O-Graph (I.E.M. GmbH, Stolberg, Germany). The cuff size was fitted according the circumference of the upper arm. Mean blood pressure was calculated and systolic and diastolic blood pressure z-scores were determined according to reference values related to height and gender (25).

Statistical analyses

Statistical analyses were performed with IBM SPSS Statistics Version 20.0. The Shapiro-Wilk test was used to test normality and Levene's test was used to test for the equality of variances. Normally distributed data are presented as mean ± standard deviation, whereas skewed data are reported as median (minimum – maximum). Comparisons between different groups were made with Pearson's chi-square test, Fisher's exact test, the independent t-test, the Mann-Whitney U test, the paired-samples t-test, the Wilcoxon

signed-rank test and the McNemar test as appropriate. All *P*-values are two-tailed and a *P*-value below 0.05 was considered statistically significant.

ClinicalTrials.gov; Registration Number: NCT02091544. Date of registration: 19/03/2014

RESULTS

245 children with a mean age of 12.4 ± 3.3 years were included for analyses in the baseline study, as presented in Figure 6.1. Baseline characteristics are presented in Table 6.1. 40% of the subjects were boys. Mean baseline BMI z-score was 3.46 ± 0.70 . 14.3% of children were classified as overweight, 42.9% as obese, and 42.9% as morbidly obese. Glomerular hyperfiltration was present in 2% to 18% of the participants, depending of the definition and equation used. Seven participants (2.9%) presented with microalbuminuria.

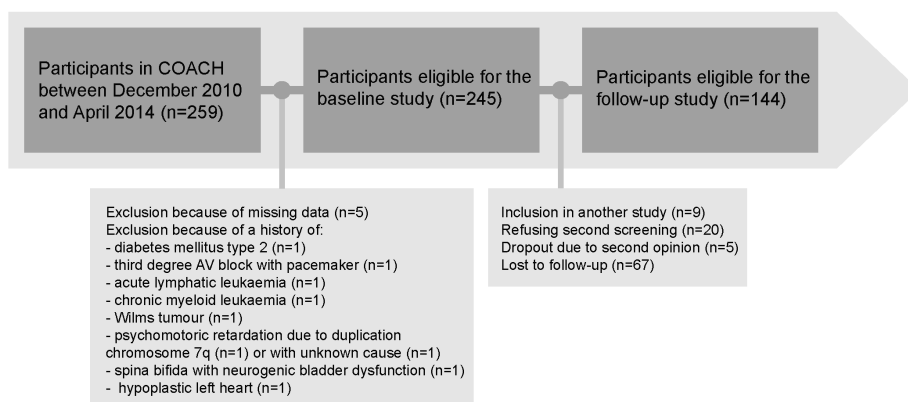


Figure 6.1. Flowchart of study inclusion. Abbreviations: COACH, Centre for Overweight Adolescent and Children's Healthcare.

At baseline, eGFR-FAS was positively correlated with eGFR-Schwartz ($r = 0.828$, $P < 0.001$, Figure 6.2A), waist-to-hip ratio ($r = 0.172$, $P = 0.010$) and triacylglyceride concentration ($r = 0.187$, $P = 0.003$). No significant correlation with fasting glucose concentration or HOMA-IR was seen. De-indexed eGFR-FAS was positively correlated with de-indexed eGFR-Schwartz ($r = 0.929$, $P < 0.001$, Figure 6.2B), age ($r = 0.729$, $P < 0.001$), BMI z-score ($r = 0.388$, $P < 0.001$), diastolic BP z-score ($r = 0.190$, $P = 0.003$), fasting glucose concentration ($r = 0.189$, $P = 0.003$), fasting insulin concentration ($r = 0.514$, $P < 0.001$), HOMA-IR ($r = 0.531$, $P < 0.001$) and triacylglyceride concentration ($r = 0.210$, $P = 0.001$). De-indexed eGFR-FAS was negatively correlated with the HDL-cholesterol concentration ($r = -0.295$, $P < 0.001$). De-indexed eGFR-Schwartz was correlated in the same manner as de-indexed eGFR-FAS with only minor differences in correlation coefficients.

Table 6.1. Baseline characteristics of the total cohort and stratified according to non-dropout versus dropout after one year lifestyle intervention.

Variable	Total (n=245)	Non-dropout (n=144)	Dropout (n=101)	P-value
Age (years)	12.4 ± 3.3	12.0 ± 3.0	13.0 ± 3.5	0.025 *
Boys (%)	40	45	34	0.09
BMI z-score	3.46 ± 0.70	3.45 ± 0.65	3.48 ± 0.78	0.76
BSA (m ²)	1.86 ± 0.46	1.81 ± 0.42	1.93 ± 0.50	0.06
Overweight (%)	14.3	11.1	18.8	0.10
Obese (%)	42.9	48.6	34.7	0.04 *
Morbid obese (%)	42.9	40.3	46.5	0.36
Prepubertal (%)	30.3	28.9	31.8	0.72
Peripubertal (%)	20.4	26.3	13.6	0.09
Postpubertal (%)	49.3	44.7	54.5	0.31
Waist circumference z-score	5.7 (1.4 - 13.9)	5.5 (1.4 - 13.9)	5.7 (1.8 - 11.5)	0.46
Hip circumference z-score	4.3 ± 2.0	4.3 ± 1.9	4.4 ± 2.2	0.60
Waist-to-hip ratio	0.93 ± 0.09	0.93 ± 0.07	0.93 ± 0.10	0.99
Systolic BP z-score	0.3 ± 1.1	0.3 ± 1.0	0.3 ± 1.2	0.91
Diastolic BP z-score	-0.4 ± 1.2	-0.4 ± 1.0	-0.4 ± 1.4	0.92
eGFR-Schwartz (ml/min/1.73m ²)	117.4 ± 20.5	119.6 ± 19.9	114.3 ± 21.1	0.06
eGFR-Schwartz > 135 ml/min/1.73m ² (%)	18.0	22.2	12.0	0.04 *
eGFR-Schwartz > 140 ml/min/1.73m ² (%)	12.7	15.3	8.9	0.17
eGFR-Schwartz > 150 ml/min/1.73m ² (%)	5.7	6.9	4.0	0.41
eGFR-Schwartz de-indexed (ml/min)	124.2 ± 30.8	123.5 ± 28.0	125.2 ± 34.4	0.66
Serum creatinine (mg/dL) / Q value	0.97 ± 0.15	0.96 ± 0.14	0.98 ± 0.16	0.32
eGFR-FAS (ml/min/1.73m ²)	113.3 ± 17.1	113.9 ± 16.3	112.3 ± 18.3	0.47
eGFR-FAS > 135 ml/min/1.73m ² (%)	11.4	13.0	9.9	0.68
eGFR-FAS > 140 ml/min/1.73m ² (%)	6.9	6.9	6.9	0.99
eGFR-FAS > 150 ml/min/1.73m ² (%)	2.0	1.4	3.0	0.41
eGFR-FAS de-indexed (ml/min)	121.4 ± 34.6	118.9 ± 30.5	124.9 ± 39.5	0.18
UACR (mg albumin / g creatinine)	4.4 (0 - 203.3)	4.0 (0.0 - 36.2)	4.4 (0.0 - 203.3)	0.10
Microalbuminuria (%)	2.9	2.1	4.0	0.45
Fasting glucose (mmol/L)	4.1 (2.1 - 5.7)	4.0 (2.5 - 5.6)	4.1 (2.1 - 5.7)	0.003 *
Fasting insulin (pmol/L)	15.3 (2.0 - 111.2)	15.0 (2.0 - 111.2)	15.5 (3.0 - 76.8)	0.40
HOMA-IR	2.5 (0.4 - 19.3)	2.5 (0.4 - 19.3)	2.8 (0.5 - 18.4)	0.16
HbA1c (%)	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.5	0.25
Total cholesterol (mmol/L)	4.5 ± 0.8	4.6 ± 0.8	4.3 ± 0.9	0.03 *
HDL-cholesterol (mmol/L)	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.67
LDL-cholesterol (mmol/L)	2.7 ± 0.7	2.8 ± 0.7	2.6 ± 0.7	0.08
Triacylglycerides (mmol/L)	1.0 (0.4 - 3.7)	1.1 (0.4 - 3.7)	1.0 (0.4 - 3.1)	0.20

Abbreviations: BMI, body mass index; BSA, body surface area; BP, blood pressure; eGFR, estimated glomerular filtration rate; FAS, full age spectrum; UACR, urine albumin-to-creatinine ratio; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein.

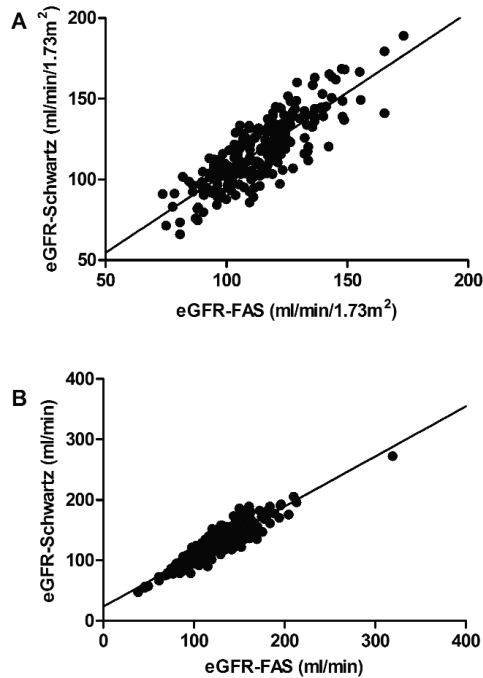


Figure 6.2. (A) Correlation between baseline eGFR-Schwartz and eGFR-FAS in ml/min/1.73m². Univariate linear regression model: $y = 4.97 + 0.99 * x$ with $R^2 = 0.685$. (B) Correlation between baseline de-indexed eGFR-Schwartz and eGFR-FAS in ml/min. Univariate linear regression model: $y = 23.74 + 0.83 * x$ with $R^2 = 0.863$.

Longitudinal study

144 participants (58.8%) had a follow-up examination. Reasons for study discontinuation are displayed in Figure 6.1. The mean interval between two assessments was 15.2 ± 3.9 months. Dropouts, as presented in Table 6.1, were significantly older than non-dropouts (13.0 ± 3.5 years versus 12.0 ± 3.0 years, $P = 0.025$) and had higher fasting glucose concentrations (4.1 mmol/L versus 4.0 mmol/L, $P = 0.003$) and lower total cholesterol concentrations (4.3 mmol/L versus 4.6 mmol/L, $P = 0.03$). eGFR-Schwartz and eGFR-FAS did not differ significantly nor did the serum creatinine / Q value ratio. Moreover, the percentage of participants with glomerular hyperfiltration did not differ significantly, except when the definition of an eGFR of more than $135 \text{ ml/min/1.73m}^2$ was used using the Schwartz equation.

Measurements before and after approximately one year lifestyle intervention as well as the mean of paired differences are presented in Table 6.2.

Table 6.2. Comparison of pre- and post-intervention data of the children with at least one year of follow-up (n=144).

Variable	Pre	Post	Mean of paired differences (pre-post)	P-value
Age (years)	12.0 ± 3.0	13.3 ± 3.0	-1.3 ± 0.3	<0.001*
BMI z-score	3.45 ± 0.65	3.28 ± 0.74	0.17 ± 0.44	<0.001*
BSA (m ²)	1.81 ± 0.42	1.92 ± 0.38	-0.11 ± 0.10	<0.001*
Normal weight (%)	0.0	1.4	-	0.50
Overweight (%)	11.1	18.1	-	0.052
Obese (%)	48.6	43.8	-	0.28
Morbid obese (%)	40.3	36.8	-	0.30
Prepubertal (%)	28.9	18.0	-	0.06
Peripubertal (%)	26.3	36.9	-	0.38
Postpubertal (%)	44.7	45.0	-	0.50
Waist circumference z-score	5.5 (1.4 - 13.9)	5.3 (1.2 - 13.8)	0.1 (-5.8 - 4.2)	0.94
Hip circumference z-score	4.3 ± 1.9	4.4 ± 1.8	-0.1 ± 0.8	0.09
Waist-to-hip ratio	0.93 ± 0.07	0.91 ± 0.08	0.02 ± 0.08	0.04*
Systolic BP z-score	0.3 ± 1.0	0.1 ± 1.2	0.2 ± 1.3	0.09
Diastolic BP z-score	-0.4 ± 1.0	-0.7 ± 1.2	0.3 ± 1.2	0.01*
Serum creatinine (mg/dL)	0.55 ± 0.13	0.61 ± 0.13	-0.06 ± 0.07	<0.001*
eGFR-Schwartz (ml/min/1.73m ²)	119.6 ± 19.9	111.6 ± 18.3	8.0 ± 15.0	<0.001*
eGFR-Schwartz > 135 ml/min/1.73m ² (%)	22.2	11.8	-	0.004*
eGFR-Schwartz > 140 ml/min/1.73m ² (%)	15.3	7.6	-	0.03*
eGFR-Schwartz > 150 ml/min/1.73m ² (%)	6.9	2.8	-	0.15
eGFR-Schwartz de-indexed (ml/min)	123.5 ± 28.0	122.5 ± 25.6	0.9 ± 17.6	0.53
Serum creatinine (mg/dL) / Q value	0.96 ± 0.14	0.99 ± 0.13	-0.03 ± 0.12	0.003*
eGFR-FAS (ml/min/1.73m ²)	113.9 ± 16.3	110.1 ± 15.1	3.8 ± 15.1	0.003*

Table 6.2. Comparison of pre- and post-intervention data of the children with at least one year of follow-up (n=144). (continued)

Variable	Pre	Post	Mean of paired differences (pre-post)	P-value
eGFR-FAS > 135 ml/min/1.73m ² (%)	13.0	5.6	-	0.03*
eGFR-FAS > 140 ml/min/1.73m ² (%)	6.9	4.2	-	0.34
eGFR-FAS > 150 ml/min/1.73m ² (%)	1.4	1.4	-	0.99
eGFR-FAS de-indexed (ml/min)	118.9 ± 30.5	122.4 ± 29.6	-3.5 ± 17.0	0.02*
UACR (mg albumin / g creatinine)	4.0 (0.0 - 36.2)	3.5 (0.0 - 401.3)	0.4 (-21.0 - 24.4)	0.86
Microalbuminuria (%)	2.1	4.3	-	0.45
Fasting glucose (mmol/L)	4.0 (2.5 - 5.6)	4.2 (2.5 - 5.3)	-0.2 (-1.3 - 1.9)	0.01*
Fasting insulin (pmol/L)	15.0 (2.0 - 111.2)	14.7 (2.1 - 64.8)	0.4 (-46.3 - 28.8)	0.19
HOMA-IR	2.5 (0.4 - 19.3)	2.7 (0.4 - 13.9)	-0.2 (-9.5 - 7.3)	0.03*
HbA1c (%)	5.3 ± 0.4	5.2 ± 0.3	0.2 ± 0.3	<0.001*
Total cholesterol (mmol/L)	4.6 ± 0.8	4.3 ± 0.8	0.3 ± 0.6	<0.001*
HDL-cholesterol (mmol/L)	1.2 ± 0.3	1.2 ± 0.3	0.0 ± 0.2	0.22
LDL-cholesterol (mmol/L)	2.8 ± 0.7	2.6 ± 0.7	0.2 ± 0.6	<0.001*
Triacylglycerides (mmol/L)	1.1 (0.4 - 3.7)	1.0 (0.4 - 3.8)	0.1 (-1.9 - 1.7)	0.04*

Abbreviations: BMI, body mass index; BSA, body surface area; BP, blood pressure; eGFR, estimated glomerular filtration rate; FAS, full age spectrum; UACR, urine albumin-to-creatinine ratio; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein.

BMI-z score, waist-to-hip ratio, diastolic BP z-score, HbA1c and concentrations of total cholesterol, LDL-cholesterol and triacylglyceride all decreased significantly after intervention. eGFR-Schwartz and eGFR-FAS decreased significantly as well. However, de-indexed eGFR-Schwartz and eGFR-FAS did not. De-indexed eGFR-FAS even increased significantly after intervention, as did the BSA, serum creatinine / Q value, fasting glucose concentration and the HOMA-IR.

Though, in the subgroup of participants with baseline glomerular hyperfiltration de-indexed eGFR changed significantly after intervention, depending on the definition for glomerular hyperfiltration and eGFR equation used; the results are displayed in Table 6.3 and 6.4.

A subgroup that started the intervention as postpubertal adolescents also showed a significant decrease in de-indexed eGFR-Schwartz (mean paired difference 9.3 ± 14.9 ml/min, 95% confidence interval (CI) 4.0-14.5). De-indexed eGFR-FAS did not decrease significantly in this cohort (mean paired difference 3.7 ± 16.8 ml/min, 95% confidence interval (CI) -2.2 – 9.5).

Analysis of post-treatment parameters demonstrated a correlation between eGFR-FAS and eGFR-Schwartz ($r = 0.726$, $P < 0.001$), fasting insulin concentration ($r = 0.171$, $P = 0.049$) and triacylglyceride concentration ($r = 0.184$, $P = 0.027$). De-indexed eGFR-FAS was positively correlated with de-indexed eGFR-Schwartz ($r = 0.872$, $P < 0.001$), age ($r = 0.645$, $P < 0.001$), BMI z-score ($r = 0.419$, $P < 0.001$), systolic BP z-score ($r = 0.221$, $P = 0.008$), diastolic BP z-score ($r = 0.289$, $P = 0.001$), fasting insulin concentration ($r = 0.374$, $P < 0.001$), HOMA-IR ($r = 0.355$, $P < 0.001$) and triacylglyceride concentration ($r = 0.259$, $P = 0.001$). De-indexed eGFR-FAS was negatively correlated with HDL-cholesterol concentration ($r = -0.276$, $P = 0.001$). De-indexed eGFR-Schwartz was correlated in the same manner as de-indexed eGFR-FAS with only minor differences in correlation coefficients. No associations of changes in de-indexed eGFR with changes in BMI z-score, blood pressure or parameters of glucose and lipid metabolism were found.

Table 6.3. Comparison of pre- and post-intervention eGFR-Schwartz stratified according to baseline glomerular hyperfiltration per definition.

Definition of glomerular hyperfiltration (n, percentile of 144 participants)	Pre eGFR-Schwartz (ml/min)	Post eGFR-Schwartz (ml/min)	Mean of paired differences (pre-post)	95% confidence interval	P-value
eGFR-Schwartz > 135 ml/min/1.73m ² (n=32, 22.2%)	139.0 ± 32.1	128.1 ± 30.5	10.8 ± 20.6	3.4 - 18.3	0.006*
eGFR-Schwartz > 140 ml/min/1.73m ² (n=22, 15.3%)	138.1 ± 32.0	128.8 ± 33.9	9.3 ± 15.4	2.5 - 16.1	0.010*
eGFR-Schwartz > 150 ml/min/1.73m ² (n=10, 6.9%)	154.3 ± 28.4	140.9 ± 30.2	13.4 ± 14.6	3.0 - 23.8	0.017*
eGFR-FAS > 135 ml/min/1.73m ² (n=18, 12.5%)	142.7 ± 29.2	129.3 ± 31.7	13.3 ± 15.8	5.5 - 21.2	0.002*
eGFR-FAS > 140 ml/min/1.73m ² (n=10, 6.9%)	153.5 ± 29.4	142.6 ± 31.5	10.9 ± 19.2	-2.8 - 24.6	0.105
eGFR-FAS > 150 ml/min/1.73m ² (n=2, 1.4%)	141.1 ± 8.9	126.9 ± 33.2	14.2 ± 24.3	n/a	n/a

Abbreviations: eGFR, estimated glomerular filtration rate; n/a, not applicable.

Table 6.4. Comparison of pre- and post-intervention eGFR-FAS stratified according to baseline glomerular hyperfiltration per definition.

Definition of glomerular hyperfiltration (n, percentile of 144 participants)	Pre eGFR-FAS (ml/min)	Post eGFR-FAS (ml/min)	Mean of paired differences (pre-post)	95% confidence interval	P-value
eGFR-Schwartz > 135 ml/min/1.73m ² (n=32, 22.2%)	127.1 ± 31.0	119.2 ± 29.0	7.9 ± 16.9	1.8 - 14.0	0.013*
eGFR-Schwartz > 140 ml/min/1.73m ² (n=22, 15.3%)	123.6 ± 28.3	117.2 ± 30.9	6.5 ± 13.4	0.5 - 12.4	0.035*
eGFR-Schwartz > 150 ml/min/1.73m ² (n=10, 6.9%)	135.6 ± 24.1	126.3 ± 26.9	9.3 ± 14.8	-1.3 - 19.8	0.079
eGFR-FAS > 135 ml/min/1.73m ² (n=18, 12.5%)	136.8 ± 30.6	126.0 ± 34.9	10.7 ± 16.1	2.8 - 18.7	0.011*
eGFR-FAS > 140 ml/min/1.73m ² (n=10, 6.9%)	148.5 ± 32.6	140.5 ± 37.7	8.0 ± 19.6	-6.0 - 22.0	0.227
eGFR-FAS > 150 ml/min/1.73m ² (n=2, 1.4%)	130.7 ± 9.1	116.5 ± 31.6	14.2 ± 22.5	n/a	n/a

Abbreviations: eGFR, estimated glomerular filtration rate; n/a, not applicable.

DISCUSSION

To the best of our knowledge, this prospective study is the first to evaluate the effect of a multidisciplinary lifestyle intervention on renal function in children and adolescents with overweight, obesity and morbid obesity without a history of renal disease. Depending on different definitions and on the equation used, glomerular hyperfiltration was present in 2% to 18% of the participants. In the total cohort of children, one year of lifestyle intervention did not significantly change eGFR after de-indexing for the BSA. However, in children with glomerular hyperfiltration at the start of the intervention, a significant decrease in eGFR was seen also after de-indexing eGFR. This decrease in eGFR was dependent on the eGFR equation and definition for glomerular hyperfiltration used.

Since measured GFR is lacking in this study, two different equations to estimate GFR were used. Though eGFR-Schwartz and eGFR-FAS correlated well, eGFR according to the Schwartz equation was approximately 5 ml/min/1.73m² higher compared to eGFR according to the FAS equation (see Figure 6.2A). After intervention, de-indexed eGFR-Schwartz did not change significantly in the total group of children. De-indexed eGFR-FAS, however, increased significantly after intervention with a mean of 3.5 ml/min. Since the serum creatinine / Q value increases, it is plausible that this post-intervention increase in de-indexed eGFR-FAS can be explained by an increase in BSA.

All current eGFR equations have been designed for GFR indexed with BSA (15). The convention of indexing GFR for BSA is routine practice since it allows direct comparison of GFR values between patients with different body sizes, and it helps in defining values that are considered as normal. Though indexation of the GFR by itself is theoretically questionable, in patients with unusual anthropometry the consequences of indexation GFR for the BSA is more important (26). In case of obesity, indexation of GFR results in a substantially lower GFR than un- or de-indexed GFR. Another argument not to index GFR in case of obesity is that the number of nephrons does not increase with increased body fat, and by indexing GFR for BSA, one can 'mask' the hyperfiltrative state (26). By using de-indexed eGFR in the longitudinal study bias due to possible weight, length or BSA change was avoided.

The pathogenesis of glomerular hyperfiltration in obesity is rather complex and not completely understood. It includes hormonal factors, including several adipokines, overactivity of the renin-angiotensin system and the renal sympathetic nervous system among others. Besides, insulin resistance has been independently associated with glomerular hyperfiltration. More specific, insulin resistance of podocytes is associated with lipid accumulation in the kidney, so called 'fatty kidneys' (2). In diabetes, glomerular hyperfiltration is mediated by the hyperglycaemic period over time (27). In youth-onset

type 2 diabetes, the most important risk factor for glomerular hyperfiltration is insulin resistance (28).

In the present study, de-indexed eGFR was positively correlated with the BMI-z score and HOMA-IR. In case of high levels of glucose over time, the glucose concentration in the ultrafiltrate in the proximal tubule increases. This induces the expression of the sodium-glucose co-transporter 2 (SGLT2), which enhances reabsorption of glucose and sodium. The increased sodium reabsorption reduces the sodium concentration in the macula densa lining the distal tubule resulting in dilatation of the afferent arteriole and production of renin. Therefore, glucose might indirectly induce glomerular hyperfiltration (29). Our group previously showed that hyperglycaemic glucose excursions are frequently observed in children with overweight and obesity (30). Future studies should focus on the association between insulin resistance, the amount of time in a hyperglycaemic state and glomerular hyperfiltration in children and adolescents with overweight and obesity.

Almost 3% of the children presented with microalbuminuria at baseline, which is in line with other studies in children with obesity (31). After lifestyle intervention, no difference in the percentages of children with microalbuminuria was noticed, which is in contrast to findings in obese adults where the urinary albumin to creatinine ratio decreased significantly as a result of weight loss (32, 33). This discrepancy might be due to an earlier, non-albuminuric phase of obesity-related renal damage present in children as compared to adults.

The study has some limitations. First, in this study, GFR was estimated using the Schwartz and FAS equation instead of directly measured. Alternative methods to estimate or determine renal function such as 24 hours urinary creatinine clearance, inulin or iothexol clearance were considered too invasive. Second, a random urine spot (RUS) was used for the estimation of the albumin excretion rate, whereas a 24 hours urine collection is considered the gold standard for the determination of albuminuria (34).

Strengths of this study include the large study population and the prospective design. Moreover, because this outpatient lifestyle intervention program caused modifications in anthropometric, cardiovascular and metabolic parameters, it has been possible to correlate these alterations with changes in renal function.

In conclusion, this study demonstrates that in children and adolescents with overweight, obesity and morbid obesity with glomerular hyperfiltration, one year of lifestyle intervention leads to a significant decrease in eGFR. This finding, however, is dependent on the eGFR equation, the definition of glomerular hyperfiltration, and on whether eGFR is de-indexed using the body surface area. Further research should focus on the associated mechanisms and clinical implications.

REFERENCES

1. Kanasaki K, Kitada M, Kanasaki M, Koya D. The biological consequence of obesity on the kidney. *Nephrol Dial Transplant*. 2013;28 Suppl 4:iv1-7
2. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol*. 2016;12(8):453-71
3. Tsuboi N, Okabayashi Y, Shimizu A, Yokoo T. The Renal Pathology of Obesity. *Kidney Int Rep*. 2017;2(2):251-60
4. Kovesdy CP, Furth SL, Zoccali C. Obesity and kidney disease: hidden consequences of the epidemic. *Kidney Int*. 2017;91(2):260-2
5. Adelman RD, Restaino IG, Alon US, Blowey DL. Proteinuria and focal segmental glomerulosclerosis in severely obese adolescents. *J Pediatr*. 2001;138(4):481-5
6. Park M, Yoon E, Lim YH, Kim H, Choi J, Yoon HJ. Renal hyperfiltration as a novel marker of all-cause mortality. *J Am Soc Nephrol*. 2015;26(6):1426-33
7. Bolignano D, Zoccali C. Effects of weight loss on renal function in obese CKD patients: a systematic review. *Nephrol Dial Transplant*. 2013;28 Suppl 4:iv82-98
8. Rijks JM, Plat J, Mensink RP, Dorenbos E, Buurman WA, Vreugdenhil AC. Children With Morbid Obesity Benefit Equally as Children With Overweight and Obesity From an Ongoing Care Program. *J Clin Endocrinol Metab*. 2015;100(9):3572-80
9. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes*. 2012;7(4):284-94
10. Fredriks AM, van Buuren S, Fekkes M, Verloove-Vanhorick SP, Wit JM. Are age references for waist circumference, hip circumference and waist-hip ratio in Dutch children useful in clinical practice? *Eur J Pediatr*. 2005;164(4):216-22
11. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child*. 1969;44(235):291-303
12. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child*. 1970;45(239):13-23
13. Susman EJ, Houts RM, Steinberg L, Belsky J, Cauffman E, Dehart G, et al. Longitudinal development of secondary sexual characteristics in girls and boys between ages 9 1/2 and 15 1/2 years. *Arch Pediatr Adolesc Med*. 2010;164(2):166-73
14. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20(3):629-37
15. Pottel H. Measuring and estimating glomerular filtration rate in children. *Pediatr Nephrol*. 2017;32(2):249-63
16. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aissa A, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant*. 2014;29(5):1082-91
17. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr*. 1978;93(1):62-6
18. Correia-Costa L, Schaefer F, Afonso AC, Bustorff M, Guimaraes JT, Guerra A, et al. Normalization of glomerular filtration rate in obese children. *Pediatr Nephrol*. 2016;31(8):1321-8
19. Cachat F, Combescure C, Cauderay M, Girardin E, Chehade H. A systematic review of glomerular hyperfiltration assessment and definition in the medical literature. *Clin J Am Soc Nephrol*. 2015;10(3):382-9
20. Piepsz A, Tondeur M, Ham H. Revisiting normal (51)Cr-ethylenediaminetetraacetic acid clearance values in children. *Eur J Nucl Med Mol Imaging*. 2006;33(12):1477-82

21. Bjornstad P, Nehus E, El Ghormli L, Bacha F, Libman IM, McKay S, et al. Insulin Sensitivity and Diabetic Kidney Disease in Children and Adolescents With Type 2 Diabetes: An Observational Analysis of Data From the TODAY Clinical Trial. *Am J Kidney Dis.* 2018 Jan;71(1):65-74
22. Braat E, Hoste L, De Waele L, Gheysens O, Vermeersch P, Goffin K, et al. Renal function in children and adolescents with Duchenne muscular dystrophy. *Neuromuscul Disord.* 2015;25(5):381-7
23. Pottel H, Mottaghy FM, Zaman Z, Martens F. On the relationship between glomerular filtration rate and serum creatinine in children. *Pediatr Nephrol.* 2010;25(5):927-34
24. Donaghue KC, Chiarelli F, Trotta D, Allgrove J, Dahl-Jorgensen K. Microvascular and macrovascular complications associated with diabetes in children and adolescents. *Pediatr Diabetes.* 2009;10 Suppl 12:195-203
25. Wuhl E, Witte K, Soergel M, Mehls O, Schaefer F, German Working Group on Pediatric H. Distribution of 24-h ambulatory blood pressure in children: normalized reference values and role of body dimensions. *J Hypertens.* 2002;20(10):1995-2007
26. Delanaye P, Radermecker RP, Rorive M, Depas G, Krzesinski JM. Indexing glomerular filtration rate for body surface area in obese patients is misleading: concept and example. *Nephrol Dial Transplant.* 2005;20(10):2024-8
27. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *J Am Soc Nephrol.* 2017;28(4):1023-39
28. Bjornstad P, Cherney DZ. Renal Hyperfiltration in Adolescents with Type 2 Diabetes: Physiology, Sex Differences, and Implications for Diabetic Kidney Disease. *Curr Diab Rep.* 2018;18(5):22
29. Anguiano Gomez L, Lei Y, Kumar Devarapu S, Anders HJ. The diabetes pandemic suggests unmet needs for 'CKD with diabetes' in addition to 'diabetic nephropathy'-implications for pre-clinical research and drug testing. *Nephrol Dial Transplant.* 2018 Aug 1;33(8):1292-1304
30. Rijks J, Karnebeek K, van Dijk JW, Dorenbos E, Gerver WJ, Stouthart P, et al. Glycaemic Profiles of Children With Overweight and Obesity in Free-living Conditions in Association With Cardiometabolic Risk. *Sci Rep.* 2016;6:31892
31. Lurbe E, Torro MI, Alvarez J, Aguilar F, Fernandez-Formoso JA, Redon J. Prevalence and factors related to urinary albumin excretion in obese youths. *J Hypertens.* 2013;31(11):2230-6;
32. Ezequiel DG, Costa MB, Chaoubah A, de Paula RB. Weight loss improves renal hemodynamics in patients with metabolic syndrome. *J Bras Nefrol.* 2012;34(1):36-42
33. Straznicky NE, Grima MT, Lambert EA, Eikelis N, Dawood T, Lambert GW, et al. Exercise augments weight loss induced improvement in renal function in obese metabolic syndrome individuals. *J Hypertens.* 2011;29(3):553-64
34. American Diabetes A. Standards of medical care in diabetes--2011. *Diabetes Care.* 2011;34 Suppl 1:S11-61

7

The evaluation of kidney function estimation during lifestyle intervention in children with overweight and obesity

Mark J.C.M. van Dam, Hans Pottel, Pierre Delanaye, Anita C.E. Vreugdenhil

Pediatr Nephrol. 2024 Nov;39(11):3271-3278

ABSTRACT

Background. Children with overweight and obesity are at risk for developing chronic kidney disease (CKD). During lifestyle adjustment, the first step in the treatment of childhood obesity, body proportions are likely to change. The aim of this study was to examine how lifestyle intervention affects creatinine-based kidney function estimation in children with overweight and obesity.

Methods. This longitudinal lifestyle intervention study included 614 children with overweight and obesity (mean age 12.17 ± 3.28 years, 53.6% female, mean BMI z-score 3.32 ± 0.75). Loss to follow-up was present: 305, 146, 70, 26 and 10 children were included after 1, 2, 3, 4 and 5 (about yearly) follow-up visits respectively. Serum creatinine (SCr) was rescaled using Q-age and Q-height polynomials.

Results. At baseline, 95-97% of the children had a SCr/Q-height and SCr/Q-age in the normal reference range of [0.67 – 1.33]. SCr/Q significantly increased each (about yearly) follow-up visit, linear mixed regression analyses demonstrated slopes between 0.01-0.04 (corresponding with eGFR FAS reduction of 1.1-4.1 ml/min/1.73m²) per visit. BMI z-score reduced in both sexes and this reduction is significantly higher in males. No correlation between change in rescaled SCr and BMI z-score reduction could be demonstrated.

Conclusions. Rescaled serum creatinine (SCr/Q) slightly increases during multidisciplinary lifestyle intervention in this cohort of children with overweight and obesity. This effect seems to be independent from change in BMI z-score. Whether this minor decrease in estimated kidney function has clinical consequences on the long-term, remains to be awaited in trials with a longer period of follow-up.

INTRODUCTION

In the last few decades, it has become widely accepted that obesity is harmful for the kidneys (1). Obesity is known to aggravate primary kidney diseases, both congenital and acquired (2-4). Besides, obesity is an independent risk factor for chronic kidney disease (CKD) in patients with no underlying kidney disease (5) and obesity-related glomerulopathy is a distinct proteinuric kidney disease with its own histological characteristics (6, 7). A hallmark of obesity-related kidney disease is glomerular hyperfiltration, a state of increased glomerular filtration rate (GFR). This hyperfiltration can be 'absolute', in case of a normal number of functioning nephrons, or 'relative', in case of congenital or acquired reduced number of functioning nephrons (8). A high GFR is often used as a surrogate marker for hyperfiltration, a controversial topic since it is still impossible to exactly determine nephron mass in a clinical setting (9).

Serum creatinine (SCr) is the endogenous marker of first choice to estimate kidney function. Although its properties make it an almost ideal marker, multiple factors can influence SCr apart from kidney function, such as muscle mass (10). SCr during childhood is heavily age-dependent, because levels of SCr increase with muscle mass and thus with age and height. Between the age of 2 and 14 years, SCr increases in a linear way with no differences between sexes. Thereafter, on average, SCr increases in females to a level of 0.7 mg/dL and in males to 0.9 mg/dL at the age of 18 years (11).

Most children with overweight and obesity without known kidney disease have a SCr within the normal reference range for children (12) and it is known that the choice of SCr-based GFR-estimating equation has an enormous impact on the value of estimated GFR (eGFR) (13). During a lifestyle intervention program, especially when this includes increased physical activity, body proportions (e.g. fat and muscle mass ratio) are likely to change and it is therefore plausible that SCr will change as well. Since increased physical activity leads to more muscle mass, SCr might increase as a result.

Lifestyle adjustment is the first step in the treatment of obesity in both children and adults, and data how lifestyle intervention affects serum creatinine and thus kidney function estimation in children is lacking. Therefore, the goals of this study are:

- to evaluate the course of SCr levels during a lifestyle intervention in children with overweight and obesity;
- to examine what happens with rescaled SCr for sex and age or height (SCr/Q-age and SCr/Q-height respectively);
- to examine whether all of these findings are different in children stratified according to BMI z-score response during the lifestyle intervention program.

METHODS

Setting and study inclusion

For this study, both baseline and follow-up data were used from children participating in the Centre for Overweight Adolescent and Children's Healthcare (COACH) at Maastricht University Medical Centre + (MUMC+) MosaKids Children's Hospital. COACH offers children and their families a personalized multidisciplinary lifestyle intervention with monthly evaluations at the outpatient department of pediatricians, physiotherapists, dieticians and social workers among others, as described previously (14). Both at baseline and about yearly (not necessarily every 12 months, median 14.6 months (interquartile range 12.7 – 17.5 months) in between baseline and first follow-up moment), children are evaluated both clinically and by means of laboratory tests among others. Between January 1st 2011 and April 1st 2019 662 children entered the program. Excluded for this study were children with secondary causes of overweight, congenital or acquired kidney disease, diabetes mellitus and/or current use of antihypertensive medication ($n=13$). Twenty-five children were excluded because of missing data. Five participants were older than 18 years at the moment of clinical evaluation and 5 children did not meet the criteria for overweight or obesity. Finally, 614 children were included in the baseline study. Loss to follow-up was present and not all children who continued the lifestyle intervention program did agree with yearly blood analyses and were therefore considered lost to follow-up for this particular study. Therefore in this longitudinal study, 305, 146, 70, 26 and 10 children were included after 1, 2, 3, 4 and 5 years respectively, presented in Figure 7.1. This study was approved by the Medical Ethical Committee of the MUMC+ and met the guidelines administered by the Declaration of Helsinki. Informed consent was obtained from all children 12 years or older, and from all parents or legal guardians.

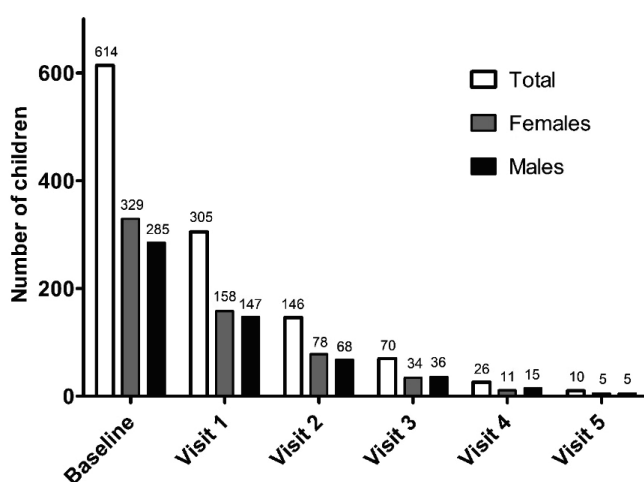


Figure 7.1. Number of children at baseline and during the follow-up study, displayed for both females and males.

Clinical assessment and anthropometry

Both at baseline and about yearly, children underwent physical examination and measurement of weight and height (using a digital scale (Seca, Chino, CA) and digital stadiometer (De Grood Metaaltechniek, Nijmegen, the Netherlands) respectively). Overweight, obesity and severe obesity were defined according to the International Obesity Task Force (IOTF) criteria (15). Clinically relevant BMI z-score reduction was defined as a Δ BMI z-score (BMI z-score baseline minus BMI z-score of last visit) of 0.25 and 0.50 (16). The equation by Haycock et al. was used to calculate body surface area (BSA) out of height (cm) and weight (kg), $BSA = 0.024265 \times Wt^{0.5378} \times Ht^{0.3964}$ (17). In a subgroup of randomly selected children (n=291, 88, 50, 25, 7 and 0 at baseline, follow-up moment 1, 2, 3, 4 and 5 respectively; data was insufficient for paired analyses), fat and fat-free mass were determined using air displacement plethysmography (BodPod).

Serum creatinine (SCr), rescaling of SCr for sex and age or height and SCr-based eGFR

The enzymatic method (Cobas 8000, Roche) was used to determine serum creatinine (SCr). Since SCr during childhood is heavily dependent on age and height (and sex from the age of 14 years), SCr was normalized using references values obtained from the literature (11, 18), resulting in Q-age and Q-height, in which Q is the median SCr for healthy children according to sex and age (Q-age) (19) or height (Q-height) (18). SCr/Q is expected to be '1' for healthy children, and values of 0.67 and 1.33 represent the 2.5th and 97.5th percentile of the normal reference range (20). SCr was converted into eGFR using the following equations: full-age spectrum (FAS), both height-independent (FAS-age) as height-dependent (FAS-height) (18, 19); new European Kidney Function Consortium (EKFC) (21); updated bedside Schwartz or CKiD (22); Schwartz-Lyon (23); CKiD under 25 years (CKiDU25) (24); adjusted-creatinine revised Lund-Malmö (LMR18) (25); and CKD-EPI equation with age-adjusted creatinine values (CKD-EPI40) (26).

Statistical analysis

Summary statistics are presented as mean $\pm$ standard deviation (for normally distributed data) and median (interquartile range) otherwise. Delta of paired variables was calculated out of value of the last visit minus baseline value. Comparisons between groups were made with the independent samples t-test for normally distributed data and the independent samples Mann-Whitney U test otherwise. All P-values are two-tailed and a P-value below 0.05 was considered statistically significant. Statistics were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Clinical Trial registration. ClinicalTrial.gov; Registration Number: NCT02091544.

RESULTS

Baseline

At baseline, 614 children were included of whom 329 were female (53.6%) and 285 were male (46.4%), demonstrated in Table 7.1. Mean age was 12.17 ± 3.28 years and mean BMI z-score was 3.32 ± 0.75 . Overweight, obesity and severe obesity was present in 128 (20.8%), 274 (44.6%) and 212 (34.5%) of the children respectively. Mean percentage of fat mass of total body weight was 44.15 ± 6.37 percent (n=291). Mean SCr/Q-age was 0.99 ± 0.16 and SCr/Q-height was 0.96 ± 0.17 . Of the children, 593 (96.6%) and 582 (94.8%) had a SCr/Q-age and SCr/Q-height in the normal reference range of [0.67 - 1.33]. Mean eGFR was in between 95.4 - 113.4 ml/min/1.73m² and 101.9 - 118.6 ml/min/1.73m² for females and males respectively, depending on the eGFR equation used.

Table 7.1. Baseline characteristics of the children with overweight and obesity stratified according to sex.

Variable	Total cohort (n=614)	Female (n=329)	Male (n=285)	P-value
Age (years)	12.17 ± 3.28	12.41 ± 3.43	11.90 ± 3.09	0.027*
Weight (kg)	73.1 ± 27.7	73.2 ± 27.6	73.1 ± 27.8	0.481
Height (cm)	154.0 ± 17.0	153.1 ± 16.3	155.0 ± 17.7	0.091
BSA (m ²)	1.78 ± 0.43	1.77 ± 0.43	1.78 ± 0.43	0.398
BMI z-score	3.32 ± 0.75	3.14 ± 0.70	3.52 ± 0.76	<0.001*
Fat mass (% of total body weight) ¹	44.15 ± 6.37	43.69 ± 6.35	44.62 ± 6.38	0.107
SCr (mg/dL)	0.58 ± 0.14	0.58 ± 0.13	0.58 ± 0.14	0.271
SCr/Q-age	0.99 ± 0.16	1.01 ± 0.15	0.97 ± 0.15	<0.001*
SCr/Q-height	0.96 ± 0.17	0.98 ± 0.17	0.93 ± 0.15	<0.001*
eGFR FAS-age	111.0 ± 18.1	108.6 ± 17.0	113.8 ± 19.4	<0.001*
eGFR FAS-height	115.8 ± 20.6	113.4 ± 20.9	118.6 ± 20.2	<0.001*
eGFR EKFC	103.9 ± 11.1	101.4 ± 11.7	106.8 ± 10.5	<0.001*
eGFR CKiD ('bedside Schwartz')	113.8 ± 20.0	112.5 ± 20.3	115.3 ± 19.6	0.043*
eGFR Schwartz-Lyon	103.5 ± 17.6	100.2 ± 18.1	107.3 ± 17.0	<0.001*
eGFR CKiD-U25	106.6 ± 17.0	100.8 ± 16.4	113.2 ± 17.8	<0.001*
eGFR LMR18	98.4 ± 10.9	95.4 ± 10.8	101.9 ± 11.0	<0.001*
eGFR CKD-EPI40	100.4 ± 13.2	96.6 ± 13.8	104.7 ± 12.5	<0.001*

Data presented as mean $\pm$ standard deviation. Abbreviations: BSA, body surface area; BMI, body mass index; SCr, serum creatinine; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials; FAS, full-age spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25; LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children. ¹ Data was available for 291 children (147 females, 144 males). P value is obtained with the independent samples t-test. A P value below 0.05 was considered statistically significant.

Compared to females, males were slightly younger (12.41 years versus 11.90 years, $p = 0.027$), had a higher BMI z-score (3.14 versus 3.52, $p < 0.001$) and significantly lower SCr/Q and higher eGFR.

Follow-up

Loss to follow-up was present and not all children who continued in the lifestyle intervention program did agree with yearly blood analyses. Therefore, in the longitudinal study, 305, 146, 70, 26 and 10 children were included after 1, 2, 3, 4 and 5 repeated measurements respectively, as presented in Figure 7.1. Compared to children who were lost to follow-up after baseline measurement ($n=309$), children with ≥ 1 follow-up measurement(s) ($n=305$) were significantly younger at baseline (11.68 ± 3.00 years versus 12.66 ± 3.47 years, $p < 0.001$), but did not differ concerning sex, BMI z-score, SCr/Q-age and SCr/Q-height. Compared to children with exactly one follow-up measurement ($n=159$), children with ≥ 2 follow-up measurements ($n=146$) were significantly younger at the first follow-up measurement (12.63 ± 2.73 years versus 13.46 ± 3.08 years, $p = 0.014$), but did not differ concerning sex, BMI z-score, SCr/Q-age and SCr/Q-height. Compared to children with exactly 2 follow-up measurements ($n=76$), children with ≥ 3 follow-up measurements ($n=70$) did not differ concerning age, sex, BMI z-score, SCr/Q-age and SCr/Q-height. Compared to children with exactly 3 follow-up measurements ($n=44$), children with ≥ 4 follow-up measurements ($n=26$) were significantly younger at the third follow-up measurement (median 13.94 (IQR 12.68 – 15.06) years versus median 16.21 (IQR 14.67 – 17.43) years, $p < 0.001$), but did not differ concerning sex, BMI z-score, SCr/Q-age and SCr/Q-height. Compared to children with exactly 4 follow-up measurements ($n=16$), children with ≥ 5 follow-up measurements ($n=10$) did not differ concerning age, sex, BMI z-score, SCr/Q-age and SCr/Q-height during the fourth follow-up measurement.

In Table 7.2 and 7.3, data for paired variables are displayed for females and males respectively. Inherent to increasing age and the mean baseline age of 12 years of our cohort, weight and height (and thus BSA) increases with each visit in both sexes. BMI z-score decreases in both females and males and this reduction is significantly higher in males ($p < 0.05$; with the exception of visit 3 and 4 ($p = 0.06$ and 0.65 respectively)). There was a significant (linear) correlation between BMI z-score and fat mass ($r = 0.59$, $p < 0.0001$). SCr increases with each visit with no differences between sexes. As displayed in Table 7.2 and 7.3, rescaled SCr does slightly increase with each visit. Note, in healthy children, SCr/Q-age does not change with age, since the normalization with Q-age makes SCr/Q age- and sex-independent. We applied a linear mixed regression model with random intercept, with SCr/Q-age as the dependent variable and age and sex as the independent variables to investigate whether the slope differs from zero or not. The slopes for males (0.01305 , $p = 0.0006$) and for females (0.02307 , $p < 0.0001$) are significantly different from zero, with an increasing trend in SCr/Q-age for both sexes with age. When fat mass was added as an extra covariate (which seriously reduces the number of observations used, therefore not included in Table 7.2 and 7.3), this was not significant. When SCr was rescaled using Q-height polynomials, a significant interaction effect ($p < 0.0001$) arises indicating that SCr/Q-height changes differently for males and females (which is unexpected since the SCr-height curve, as defined by Hoste et al. (18), is independent of sex). In this model, the linear increase per visit in SCr/Q-height was found to be 0.04820 and 0.003301 in females and males respectively.

Table 7.2. Follow-up data of females during lifestyle intervention.

Visit	Intervention duration (months)	Δ weight (kg)	Δ height (cm)	Δ BSA (m2)	Δ BMI z-score	Δ SCr (mg/dL)	Δ SCr/Q-age	Δ SCr/Q-height
1 (n=158)	14.2 (12.7-16.8)	6.6 (2.6-10.0)	5.6 (1.4-8.5)	0.12 (0.04-0.17)	-0.04 (-0.26 -0.13)	0.04 (0.00-0.09)	0.01 (-0.06 -0.09)	0.03 (-0.06 -0.09)
2 (n=78)	28.0 (26.0-32.3)	12.5 (6.3-19.2)	11.2 (2.0-15.1)	0.24 (0.10-0.31)	-0.04 (-0.42 -0.24)	0.10 (0.05-0.16)	0.06 (-0.03 -0.16)	0.08 (0.01-0.17)
3 (n=34)	41.6 (38.8-47.6)	20.3 (10.4-29.2)	13.5 (2.8-21.5)	0.34 (0.16-0.48)	-0.14 (-0.44 -0.31)	0.18 (0.10-0.23)	0.10 (0.02-0.20)	0.12 (0.05-0.23)
4 (n=11)	60.8 (52.3-69.6)	28.6 (18.5-39.9)	27.7 (21.2-36.6)	0.55 (0.36-0.66)	-0.33 (-1.34 -0.44)	0.23 (0.12-0.26)	0.15 (-0.01 -0.24)	0.17 (0.06-0.24)
5 (n=5)	84.5 (60.6-91.1)	57.6 (38.0-61.9)	29.0 (15.4-45.4)	0.89 (0.62-1.00)	0.90 (-0.18 -1.10)	0.29 (0.16-0.34)	0.26 (0.01-0.30)	0.23 (0.08-0.30)

Data presented as median (interquartile range). Delta of paired variables was calculated out of value of the last visit minus baseline value. Abbreviations: BSA, body surface area; BMI, body mass index; SCr, serum creatinine; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials.

Table 7.3. Follow-up data of males during lifestyle intervention.

Visit	Intervention duration (months)	Δ weight (kg)	Δ height (cm)	Δ BSA (m2)	Δ BMI z-score	Δ SCr (mg/dL)	Δ SCr/Q-age	Δ SCr/Q-height
1 (n=147)	15.4 (13.0-18.6)	8.0 (2.9-13.3)	7.5 (5.2-10.7)	0.13 (0.06-0.24)	-0.25 (-0.53 -0.06)	0.06 (0.01-0.10)	0.00 (-0.06 -0.07)	0.00 (-0.07 -0.07)
2 (n=68)	29.2 (26.5-34.8)	18.7 (8.8-24.5)	16.1 (11.4-19.7)	0.32 (0.18-0.43)	-0.29 (-0.65 -0.03)	0.12 (0.08-0.19)	0.03 (-0.06 -0.11)	0.02 (-0.05 -0.09)
3 (n=36)	45.9 (41.1-59.6)	29.8 (19.6-36.7)	21.8 (17.1-28.1)	0.48 (0.34-0.60)	-0.34 (-0.65 -0.08)	0.24 (0.16-0.29)	0.10 (-0.04 -0.18)	0.06 (-0.04 -0.13)
4 (n=15)	59.8 (54.8-69.5)	37.1 (17.5-42.3)	24.3 (19.7-27.0)	0.56 (0.33-0.65)	-0.74 (-0.96 - -0.15)	0.28 (0.15-0.36)	0.10 (-0.08 -0.21)	0.10 (-0.04 -0.19)
5 (n=5)	78.2 (69.5-86.7)	35.2 (30.6-50.8)	32.3 (29.6-38.1)	0.63 (0.57-0.83)	-1.13 (-1.31 -0.22)	0.35 (0.14-0.40)	0.09 (-0.12 -0.25)	0.19 (-0.06 -0.34)

Data presented as median (interquartile range). Delta of paired variables was calculated out of value of the last visit minus baseline value. Abbreviations: BSA, body surface area; BMI, body mass index; SCr, serum creatinine; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials.

Effect of changes in BMI z-score

As shown in Table 7.2 and 7.3, during the lifestyle intervention BMI z-score decreases in both females and males. A linear mixed regression model with random intercept, with BMI z-score as the dependent variable and age and sex as the independent variables, demonstrated a significant decrease of BMI z-score in males (slope is -0.07556, $p < 0.0001$), but not in females (slope = -0.01561, $p = 0.3383$).

In order to examine associations between BMI z-score reduction and change in SCr/Q-age, 70 children with 4 consecutive visits were selected (baseline and 3 visits thereafter), displayed in Table 7.4 and 7.5 for females ($n=34$) and males ($n=36$) respectively. Of the females, 61.8%, 41.2% and 17.6% had a BMI z-score reduction of at least 0.00, 0.25 and 0.50 respectively. Of the males, 75.0%, 61.1% and 36.1% had a BMI z-score reduction of at least 0.00, 0.25 and 0.50 respectively. In both sexes, no differences between change in rescaled SCr could be demonstrated between the children who did reduce in BMI z-score and children who did not. We could not demonstrate a significant correlation between the change in BMI z-score and change in SCr/Q. In the total cohort of children (and not only in the children with 4 consecutive visits), a random intercept linear mixed effects model, using visit, sex and the interaction of visit with sex, demonstrated a significant increase (positive slope significantly different from zero) in SCr/Q-age with visit or SCr/Q-height with visit, both in females and males, but more pronounced in females. Even after adjusting for BMI-z and age, these effects remained. The same observations were made in the restricted dataset of children with 4 consecutive visits.

Table 7.4. Change in rescaled SCr after 4 consecutive visits in females (n=34), stratified according to BMI z-score reduction.

	Δ BMI z $\geq$ 0.00 (n=13)	Δ BMI z < 0.00 (n=21)	p-value	Δ BMI z > -0.25 (n=20)	Δ BMI z $\leq$ -0.25 (n=14)	p-value	Δ BMI z > -0.50 (n=28)	Δ BMI z $\leq$ -0.50 (n=6)	P-value
Δ SCr/Q-age	0.12 (0.01-0.18)	0.09 (0.02-0.24)	0.889	0.10 (-0.02 -0.19)	0.09 (0.02-0.23)	0.877	0.10 (0.06-0.21)	0.06 (0.00-0.20)	0.581
Δ SCr/Q-height	0.18 (0.04-0.26)	0.11 (0.05-0.23)	0.649	0.17 (0.05-0.26)	0.11 (0.05-0.21)	0.569	0.14 (0.05-0.24)	0.10 (0.06-0.22)	0.947

Median intervention duration 41.6 months (interquartile range 38.8-47.6 months). Data presented as median (interquartile range). Delta of paired variables was calculated out of value of the last visit minus baseline value. Abbreviations: BMI, body mass index; SCr, serum creatinine; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials. P-value is obtained with the independent samples Mann-Whitney U Test.

Table 7.5. Change in rescaled SCr after 4 consecutive visits in males (n=36), stratified according to BMI z-score reduction.

	Δ BMI z $\geq$ 0.00 (n=9)	Δ BMI z < 0.00 (n=27)	P-value	Δ BMI z > -0.25 (n=14)	Δ BMI z $\leq$ -0.25 (n=22)	P-value	Δ BMI z > -0.50 (n=23)	Δ BMI z $\leq$ -0.50 (n=13)	P-value
Δ SCr/Q-age	0.02 (-0.06 -0.20)	0.10 (-0.01 -0.18)	0.810	0.11 (-0.06 -0.19)	0.09 (-0.02 -0.19)	0.855	0.10 (-0.05 -0.18)	0.08 (0.02-0.20)	0.932
Δ SCr/Q-height	0.03 (-0.04 -0.14)	0.09 (-0.05 -0.13)	0.897	0.03 (-0.05 -0.14)	0.10 (-0.03 -0.12)	0.803	0.10 (-0.04 -0.13)	0.05 (-0.04 -0.13)	0.771

Median intervention duration 45.9 months (interquartile range 41.1-59.6 months). Data presented as median (interquartile range). Delta of paired variables was calculated out of value of the last visit minus baseline value. Abbreviations: BMI, body mass index; SCr, serum creatinine; SCr/Q-age, serum creatinine normalized using Q-age polynomials; SCr/Q-height, serum creatinine normalized using Q-height polynomials. P-value is obtained with the independent samples Mann-Whitney U Test.

DISCUSSION

The main conclusion of this study is that, even though a slightly increase in rescaled serum creatinine (SCr/Q) is observed, a clinician should not expect a major change in SCr-based estimated kidney function during lifestyle intervention in children with overweight and obesity. During this lifestyle intervention BMI z-score decreased in both females and males and this reduction is significantly higher in males. There was no correlation between change in BMI z-score and change in SCr/Q and no difference in change in SCr/Q during follow-up between children that achieved a significant reduction in BMI z-score and children who did not.

So how can we interpret the SCr/Q increase and how can this minor increase in SCr/Q be explained? As described previously (12, 13), the children in this study are not known with overt kidney disease and about 95% of the children in our cohort had a SCr/Q in the normal reference range (in other words, a normal estimated kidney function) at baseline. Assuming a normal SCr/Q (so a value of '1') and thus eGFR-FAS of 107.3 ml/min/1.73m² (since eGFR-FAS equals 107.3/(SCr/Q)), the observed about yearly SCr/Q increase of 0.01–0.04 equals an eGFR-FAS reduction of about 1.1 – 4.1 ml/min/1.73m². Knowing that SCr is a marker for both kidney function and muscle mass, this minor increase in SCr/Q over time cannot be interpreted easily and we come to the following hypotheses.

First, the fact that SCr itself increases with each visit during this lifestyle intervention program is not abnormal in children aged around 12 years old. Namely, each visit goes hand in hand with age, and increasing age means increasing muscle mass, hence, increasing SCr (11). Though, since SCr/Q is independent of sex and age (Q-age), or height (Q-height), one should not expect that an increase in SCr/Q can be explained by merely increasing age.

Second, it seems plausible that during a lifestyle intervention program, the amount of muscle mass not only increases with age, but also resulting from more physical activity. Increase in muscle mass leads to higher SCr levels and, since this effect is age- and height-independent, also increased SCr/Q. In this study, we determined the percentage of fat mass using air displacement plethysmography in a randomly selected group of children. When fat mass was added as an extra covariate (which seriously reduces the number of observations used which is a limitation of this study), a significant effect on change in SCr/Q could not be demonstrated.

Finally, there is the hyperfiltration hypothesis. Multiple studies demonstrate that obesity-related hyperfiltration might be reversible in case of weight loss (27, 28). So, one should expect that during lifestyle intervention, this is also the case in children in whom obesity-related hyperfiltration might already be present. As a result, it can be expected that this

supraphysiological GFR decreases, which corresponds with increase in SCr or SCr/Q. In the present study however, we could not demonstrate a correlation between change in BMI z-score and change in SCr/Q, nor a difference in change in SCr/Q during follow-up between children that achieved a significant reduction in BMI z-score and children who did not.

In this study, both Q-age (19), and Q-height (18) were used to rescale SCr and to correct for sex and age, and height respectively. The fact that a difference in SCr/Q evolution between males and females is demonstrated when Q-height is used as the scaling factor (something that is not seen when Q-age is used to scale SCr) is intriguing and deserves further investigation. Note that the SCr-height curve, as defined by Hoste et al. (18), is independent of sex after all.

To the best of our knowledge, the present study is the first to evaluate the course of SCr levels during a lifestyle intervention in children with overweight and obesity. However, this study is not without limitations. First, this study did not include measured GFR. Besides, GFR was estimated with SCr, though other endogenic biomarkers, such as cystatin C, could be of interest as well. Second, loss to follow-up was highly present. While this is inherent to a lifestyle intervention program, a drop out of about 50% children about each year leads to bias. Third, this study lacks a control group. We believe, though, this is only a minor limitation, since our cohort size justified stratification according to BMI z-score reduction or not, and the course of SCr in healthy children without lifestyle intervention is already known. Finally, though we included gold standard measurement of body proportions using air displacement plethysmography, due to missing data, group size significantly dropped when fat mass was added as an independent variable. As body composition is a well-known confounder of SCr, it is a serious limitation of this study that fat and fat-free mass was only measured in a minority of the children. Because of this, in line with other studies (16), effect of the lifestyle intervention was defined as BMI z-score reduction, whilst BMI z-score has serious limitations (e.g. it does not discriminate weight gain resulting from excess muscle mass or fat mass, nor does it discriminate between central and visceral adiposity).

In conclusion, this study demonstrates that rescaled serum creatinine (SCr/Q) slightly increases during lifestyle intervention in children with overweight and obesity. This effect seems to be independent from change in BMI z-score. Whether this minor change in estimated kidney function has clinical consequences on the long-term, remains to be awaited in trials with a longer period of follow-up.

REFERENCES

1. Kovesdy CP, Furth SL, Zoccali C. Obesity and kidney disease: hidden consequences of the epidemic. *Pediatr Nephrol.* 2017;32(4):537-45
2. Groen In 't Woud S, Roeleveld N, Westland R, Renkema KY, Steffens MG, Gracchi V, et al. Uncovering risk factors for kidney injury in children with a solitary functioning kidney. *Kidney Int.* 2023 Jan;103(1):156-165
3. Nowak KL, You Z, Gitomer B, Brosnahan G, Torres VE, Chapman AB, et al. Overweight and Obesity Are Predictors of Progression in Early Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol.* 2018;29(2):571-8
4. Kanbay M, Yildiz AB, Yavuz F, Covic A, Ortiz A, Siriopol D. The role of body mass index on IgA nephropathy prognosis: a systematic review and meta-analysis. *Int Urol Nephrol* 2022;54:2567–2579
5. Garofalo C, Borrelli S, Minutolo R, Chiodini P, De Nicola L, Conte G. A systematic review and meta-analysis suggests obesity predicts onset of chronic kidney disease in the general population. *Kidney Int.* 2017;91(5):1224-35
6. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol.* 2016;12(8):453-71
7. Martínez-Montoro JI, Morales E, Cornejo-Pareja I, Tinahones FJ, Fernández-García JC. Obesity-related glomerulopathy: Current approaches and future perspectives. *Obes Rev.* 2022;23(7):e13450
8. Cortinovis M, Perico N, Ruggenenti P, Remuzzi A, Remuzzi G. Glomerular hyperfiltration. *Nat Rev Nephrol.* 2022;18(7):435-51
9. Chakkeri HA, Denic A, Kremers WK, Stegall MD, Larson JJ, Ravipati H, et al. Comparison of high glomerular filtration rate thresholds for identifying hyperfiltration. *Nephrol Dial Transplant.* 2020;35(6):1017-26
10. den Bakker E, Gemke R, Bokenkamp A. Endogenous markers for kidney function in children: a review. *Crit Rev Clin Lab Sci.* 2018;55(3):163-83
11. Pottel H, Vrydags N, Mahieu B, Vandewynckele E, Croes K, Martens F. Establishing age/sex related serum creatinine reference intervals from hospital laboratory data based on different statistical methods. *Clin Chim Acta.* 2008;396(1-2):49-55
12. van Dam M, Pottel H, Vreugdenhil ACE. Creatinine-based GFR-estimating equations in children with overweight and obesity. *Pediatr Nephrol.* 2022;37(10):2393-403
13. van Dam M, Pottel H, Vreugdenhil ACE. Relation between obesity-related comorbidities and kidney function estimation in children. *Pediatr Nephrol.* 2023;38(6):1867-76
14. Karnebeek K, Thapar S, Willeboordse M, van Schayck OCP, Vreugdenhil ACE. Comorbidities in primary versus secondary school children with obesity and responsiveness to lifestyle intervention. *J Clin Endocrinol Metab* 2019;104:3803–3811
15. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes.* 2012;7(4):284-94
16. Reinehr T, Lass N, Toschke C, Rothermel J, Lanzinger S, Holl RW. Which Amount of BMI-SDS Reduction Is Necessary to Improve Cardiovascular Risk Factors in Overweight Children? *J Clin Endocrinol Metab.* 2016;101(8):3171-9
17. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr.* 1978;93(1):62-6

18. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aïssa A, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant*. 2014;29(5):1082-91
19. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol*. 2012;27(6):973-9
20. Pottel H, Hoste L, Delanaye P. Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m². *Pediatr Nephrol*. 2015;30(5):821-8
21. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, et al. Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate : A Cross-sectional Analysis of Pooled Data. *Ann Intern Med*. 2021;174(2):183-91
22. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20(3):629-37
23. De Souza VC, Rabilloud M, Cochat P, Selistre L, Hadj-Aïssa A, Kassai B, et al. Schwartz formula: is one k-coefficient adequate for all children? *PloS One*. 2012;7(12):e53439
24. Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ. Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. *Kidney Int*. 2021;99(4):948-56
25. Björk J, Nyman U, Delanaye P, Grubb A, Larsson A, Vranken L, et al. A novel method for creatinine adjustment makes the revised Lund-Malmö GFR estimating equation applicable in children. *Scand J Clin Lab Invest*. 2020;80(6):456-63
26. Björk J, Nyman U, Larsson A, Delanaye P, Pottel H. Estimation of the glomerular filtration rate in children and young adults by means of the CKD-EPI equation with age-adjusted creatinine values. *Kidney Int*. 2021;99(4):940-7
27. Chagnac A, Weinstein T, Herman M, Hirsh J, Gaftor U, Ori Y. The effects of weight loss on renal function in patients with severe obesity. *J Am Soc Nephrol*. 2003;14(6):1480-6
28. van Dam M, Rijks J, Dorenbos E, Horuz F, van Dael K, Vreugdenhil A. The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity. *Sci Rep*. 2019;9(1):4504

8

General discussion

Over the past decades, the prevalence of childhood obesity has majorly increased. Since obesity is a well-established risk factor for developing chronic kidney disease (CKD), the world is highly likely heading towards a serious CKD pandemic (1-3). Obesity thus has to be considered as an important modifiable factor in the prevention and deterioration of CKD. The main objectives of this thesis were to gain more insight into serum creatinine (SCr)-based estimated glomerular filtration rate (eGFR) in children with overweight and obesity, to gain more insight into the relation between eGFR and multiple obesity-related comorbidities, and to examine the effect of a lifestyle intervention on eGFR in children with overweight and obesity.

The studies described in this thesis all use data from participants of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at Maastricht University Medical Centre + (MUMC+) MosaKids Children's Hospital. As described in **Chapter 1**, COACH is an expertise centre for children with overweight and obesity in which a multidisciplinary lifestyle intervention program was developed with the task to improve lifestyle and obesity-related conditions of children. Whilst data were collected since 2011 and this data collection is still ongoing, for this thesis, research questions were made after most of the data used for this thesis were collected. Needless to say, this retrospective methodology has limitations and the findings of our studies have to be seen in light of this. One of these methodological limitations is the absence of a control group. The cross-sectional studies (**Chapters 2, 3, 4, and 5**) lack a reference group of children without overweight and obesity, which was inherent to the design of data collection. The longitudinal studies (**Chapters 6 and 7**) did not include a control group of children with overweight and obesity that did not receive the lifestyle intervention. Since group sizes were rather large, and results could be compared according to BMI z-score reduction in the longitudinal studies, the lack of a control group is only a minor limitation of the prospective analyses in this thesis. Moreover, it would be unethical to withhold children with overweight and obesity from a lifestyle intervention. Apart from these limitations, the COACH cohort has a number of strong and unique features. The baseline cohort size is relatively large and children underwent extensive anthropometric and metabolic evaluations. This chapter provides a reflection on the main findings, clinical implications and future perspectives.

GLOMERULAR FILTRATION RATE (GFR) IN CHILDREN WITH OVERWEIGHT AND OBESITY

The determination of GFR is a major component of kidney function monitoring. Since direct measurements of GFR are complex and invasive, very few GFR studies involving direct measurements have been conducted in healthy children (4-6). For multiple reasons, reviewed by Pottel (7), the results of Piepsz et al. (4) might be considered as the

reference work for normal GFR values in children from 0.1 year of age onwards. In the reference study among 623 children with a unilateral well-defined congenital anomaly of the kidney and urinary tract and a normal left-to-right uptake ratio (relative uptake between 45% and 55%) on DMSA scintigraphy, GFR was measured using ^{51}Cr -EDTA (4). There is no data on the BMI z-score of the children in this reference cohort. Correia-Costa et al. described 313 children with overweight and obesity aged 8-9 years old in whom GFR was 'measured' by 24-hour creatinine clearance (8). GFR was also estimated from SCr and cystatin C (CysC) using the combined Zappitelli equation. Compared to a control group of children with normal weight, mean absolute GFR (both 'measured' and estimated) was higher in children with overweight and obesity and BSA-adjusted GFR was lower. To the best of our knowledge, no mGFR studies using gold standard methods among children with overweight and obesity without overt kidney disease have been conducted to date (excluding the control group of 26 children with obesity described in the iCARE study (9), which will be described below, in which mean mGFR was $130.0 \pm 25.8 \text{ ml/min/1.73m}^2$).

In contrast, in adults with overweight and obesity, several studies on the evaluation of GFR exist (10-16). In general, there is an inconsistency between SCr-based GFR-estimations and mGFR. In addition, in case of obesity, the indexation of GFR for BSA in general underestimates GFR and multiple studies in adults evaluated the differences between adjusted and unadjusted GFR in this population (17-20). Recently, the error of SCr-based eGFR equation and the impact of BSA indexation in 944 adults with overweight and obesity (about one in three participants had no overt kidney disease) has been described (21). Mean age was 59 ± 14 years, about 70% of the adults were male, and mean BMI was $30.8 \pm 4.8 \text{ kg/m}^2$. GFR was measured by the plasma clearance of iohexol and mean mGFR was $50.7 \text{ ml/min/1.73m}^2$ (adjusted for BSA) and 59.4 ml/min (unadjusted for BSA). Thirty-three SCr-based GFR-estimating equations were evaluated. In short: frequently used equations showed that 90% of estimations ranged from -55 to +55% of mGFR, with no major differences between individuals with overweight and obesity. The proportion of eGFR results within -30 to +30% of mGFR values (P30) were 65, 71 and 79% for SCr-, CysC- and combined SCr- and CysC-based GFR-estimating equations respectively. The adjustment for BSA of mGFR or eGFR led to a relevant underestimation of GFR, reaching at least 10 mL/min in 25% of the cases. Thus, in adults with overweight and obesity, SCr- and CysC-based equations are far from optimal in reflecting mGFR.

Since SCr-based GFR-estimating equations fail to reflect mGFR in adults with overweight and obesity, this might account for children with overweight and obesity as well. Because of this, GFR measurement with iohexol was considered in our cohort. Without going too much into detail, a variety of methodologies or procedures can be used to measure GFR with iohexol (22). Different procedures have different degrees of complexity and precision and can therefore lead to slightly different results (23). Iohexol is almost always injected intravenously as a bolus either for urinary or plasma clearance determinations.

While urinary clearance may be the most physiologic and accurate method to measure the filtering capacity of the kidney, it is time consuming and prone to errors. Plasma clearance methods represent the best compromise between physiology and feasibility, both in clinical routine and research. Practically, this method involves a venipuncture for a baseline blood sample and infusion of iohexol, then venous blood timed samplings. As an alternative, finger-stick collection for dried blood spot (DBS) may be more tolerable in the pediatric population, which might be an acceptable alternative for venous blood sampling (24). Considering all of the practical issues, we considered measuring GFR with iohexol to be too invasive for the children in COACH.

Serum creatinine (SCr)

Since GFR measurement in general is considered to be too invasive and cumbersome in children, GFR is commonly estimated using serum levels of endogenous markers (25, 26). Whilst SCr does not fulfil all criteria to be an ideal endogenous marker, SCr still is the most commonly used and advised marker for GFR estimation in both children and adults (25, 27, 28). From a pragmatic point of view, SCr measurement is easy and cheap, and its measurement is analytically robust and standardized. However, SCr-based GFR estimation has limitations and non-GFR determinants can majorly influence SCr concentrations and thus eGFR. Some of these non-GFR determinants (such as muscular mass, tubular secretion, effect of diet) are especially important in the context of obesity and SCr-based eGFR studies in children with overweight and obesity have to be seen in light of these confounding factors.

Multiple studies examined SCr in children with overweight and obesity. Some studies have demonstrated similar SCr values between children with normal weight, overweight and obesity (29-32). In other studies (8, 33-35), SCr values were higher in children with overweight or obesity compared to lean controls. It is not excluded that these inconsistent results are due to confounding factors such as age, height, and sex and/or due to the heterogeneity of the methods for SCr measurements. In **Chapter 2 and 3** of this thesis, SCr and SCr-based eGFR in 600 children with overweight and obesity without CKD were described. SCr was measured using the enzymatic method, which is equivalent to the isotope dilution mass spectrometry (IDMS) gold standard methods. Of the children, 96.5% had SCr values within the SCr/Q-age reference range of [0.67-1.33] (corresponding to the 2.5th and 97.5th percentile, which equals eGFR FAS-age of about 81-160 ml/min/1.73m²). It is known that children with overweight and obesity are taller and have an increased absolute fat mass (FM) and fat-free mass (FFM) compared to age- and sex-matched normal weight children; approximately 75% of the excess weight is FM, the remainder FFM (36). Because of this, and since SCr levels are highly influenced by muscle mass (and thus FFM), which was confirmed in our cohort described in **Chapter 3**, one should expect higher SCr values in children with overweight and obesity compared to lean children. Without knowledge of mGFR or single-nephron GFR, it cannot be

determined how and to what extent glomerular hyperfiltration has a role in this. In case of glomerular hyperfiltration, one should expect lower SCr values.

Another non-GFR determinant of SCr that needs to be discussed in the context of obesity is altered tubular secretion. Creatinine is a small molecule and is freely filtered across the glomerular membrane, making glomerular filtration the principal route of eliminations. Though, creatinine is also excreted by tubular secretion, the level of which is inversely related to GFR (25). While glomerular filtration is a passive process, tubular secretion of creatinine occurs via a variety of transports, accounting for 10-40% of total creatinine excretion (37, 38). In a study among 226 adult healthy kidney donors (39), creatinine clearance systematically overestimated GFR and this overestimation was significantly and independently associated with BMI (ranging from about 6% in lean subjects to about 15% in subjects with obesity). Both a higher BMI and male sex were independent determinants of higher fractional excretion of creatinine (FE_{creat}), suggesting that higher BMI and male sex enhance tubular creatinine handling (39). This can partially be explained by the so called 'supply hypothesis'; in healthy subjects it was demonstrated that infusion of exogenous creatinine leads to increases in FE_{creat} up to 200% immediately after infusion (40, 41). This 'supply hypothesis' states that tubular secretion rate of creatinine increases with increased supply of creatinine. Since BMI was the main determinant for FE_{creat} rather than BSA for instance, specific effects of adiposity on tubular creatinine handling are plausible. Biopsy studies indeed do not only demonstrate glomerulomegaly in individuals with obesity, but also tubular changes (42). In an animal study in which mice received a basal diet or high-fat diet (HFD) (43), in HFD-induced obese mice glomerulosclerosis was observed and the mRNA expression of tubular transporters (like Oat 1, Oct2 and Mrp4) was significantly altered in the kidneys compared to controls. So, in individuals with obesity SCr levels might not only be decreased as a result of glomerular hyperfiltration, but also as a result from enhanced tubular secretion of creatinine. This might in part explain why most children with overweight and obesity demonstrate SCr value within the normal reference range (as described in **Chapter 2 and 3**) instead of increased SCr values as can be expected based on body composition. The effect of obesity on tubular secretion of creatinine is an important argument to prefer mGFR using exogenous filtration markers over endogenous clearance of creatinine, as was performed in the, to the best of our knowledge, only 'mGFR' study in children in overweight and obesity (8).

Finally, SCr reference values are derived from healthy individuals (44, 45). In both reference studies, anthropometry or body proportion was not mentioned, and therefore, the prevalence of overweight or obesity in these cohorts is not known. It is therefore conceivable that SCr reference values are different, possibly lower, in a cohort of exclusively normal weight children.

As described in **Chapter 2 and 3**, most children with overweight and obesity have an eGFR within the normal reference range. In order to translate these findings into practice, a histogram of eGFR FAS-age (based on the data of the children described in **Chapter 2 and 3**) was made, displayed in Figure 8.1.

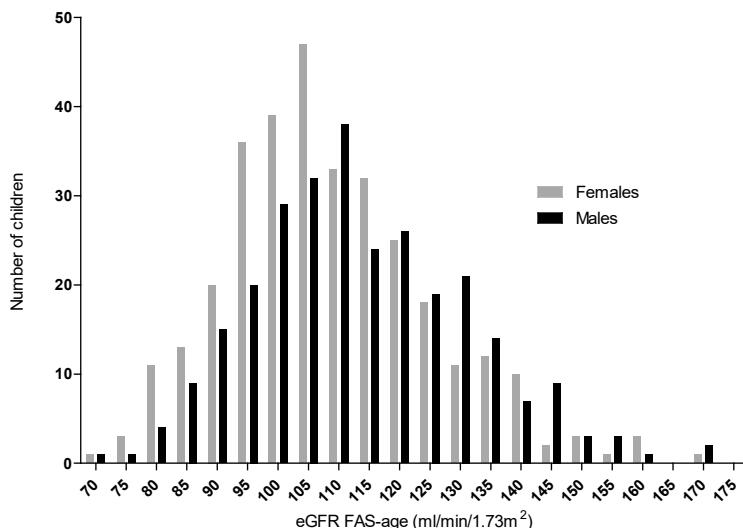


Figure 8.1. Histogram of eGFR FAS-age in the cohort of 600 children with overweight and obesity as described in Chapter 2 and 3. One child with eGFR FAS-age >175 ml/min/1.73m² was considered as an outlier and was excluded from this histogram, which therefore includes 599 children with overweight and obesity. The values on the x-axis represent eGFR categories, with each group covering an interval of 5 ml/min/1.73m². For example, the group displayed at eGFR 100 ml/min/1.73m² includes children with an eGFR ranging from 97.5 to 102.49 ml/min/1.73m². eGFR FAS-age was below 90 ml/min/1.73m² in 10.5% of the children (11.8% of the females, 9.0% of the males). eGFR FAS-age was below 80.7 ml/min/1.73m² (SCr/Q > 1.33) in 2.3% of the children (3.1% of the females, 1.4% of the males). In 3 children (0.5%; 2 females and 1 male), eGFR FAS-age was below 75 ml/min/1.73m².

According to the KDIGO 2024 CKD guideline (27), CKD stage 2 and onwards is defined as an eGFR below 90 ml/min/1.73m². In our cohort, eGFR FAS-age was below 90 ml/min/1.73m² in 10.5% of the children (11.8% of the females, 9.0% of the males). Compared to children with eGFR FAS-age ≥ 90 ml/min/1.73m², children with eGFR FAS-age below 90 ml/min/1.73m² had a significantly higher fat-free mass (58.7% versus 55.5%, $p = 0.002$) and lower waist-to-hip ratio (0.90 versus 0.93, $p = 0.006$). No differences in age or BMI z-score were demonstrated.

As described by Pottel et al. (46), the reference range for normal kidney function in healthy children is defined by SCr/Q between 0.67 and 1.33 (with lower and upper limits corresponding to the 2.5th and 97.5th percentile; where SCr/Q of 1.33 equals eGFR FAS-age of 80.7 ml/min/1.73m²). In our cohort, eGFR FAS-age was below 80.7 ml/min/1.73m²

in 2.3% of the children (3.1% of the females, 1.4% of the males). Compared to children with eGFR FAS-age ≥ 80.7 ml/min/1.73m², children with eGFR FAS-age below 80.7 ml/min/1.73m² had a significantly higher fat-free mass percentage (60.7% versus 55.8%, $p = 0.012$) and lower waist-to-hip ratio (0.88 versus 0.93, $p = 0.022$). Groups did not differ in age or BMI z-score. In only 3 children (0.5%; 2 females and 1 male) eGFR FAS-age was below 75 ml/min/1.73m² (the suggested cut-off value for CKD in children by some authors (46)).

In contrast, 9.5% of the children (7.8% of the females, 11.5% of the males) had a SCr-based eGFR FAS-age >135 ml/min/1.73m², arguably the cut-off value for glomerular hyperfiltration (47). Compared to children with eGFR FAS-age ≤ 135 ml/min/1.73m², children with assumed glomerular hyperfiltration, had a significantly lower fat-free mass percentage (52.6% versus 56.2%, $p = 0.007$) and higher waist-to-hip ratio (0.96 versus 0.92, $p < 0.001$).

Finally, in 7 children (1.2% of the children; 3 females and 4 males), eGFR FAS-age was above 160.1 ml/min/1.73m² (corresponding SCr/Q of 0.67, the suggested lower limit of normal corresponding the 2.5th percentile). Children with eGFR FAS-age below and above 160.1 ml/min/1.73m² did not differ significantly in age, BMI z-score, fat-free mass percentage and/or waist-to-hip ratio.

Serum cystatin C (CysC)

Whilst in this thesis we did not research other endogenous markers for GFR estimation besides SCr, CysC is increasingly used in practice and has advantages over SCr in specific situations. Though CysC does not obviously outperform SCr as a kidney function biomarker in individuals with obesity, it is beyond dispute that the absence of serum CysC levels is a limitation in this thesis. Here, we will describe in short the evidence for CysC-based GFR-estimation.

Cystatin C (CysC) is a low molecular weight protein and is part of the cystatin family of anti-proteinases (25) and CysC was described for the first time as a potential GFR biomarker in 1985 (48). It is presented as a biomarker for kidney function with the advantage of being not dependent (or at least much less so than SCr (49)) on muscular mass (50, 51). Though, serum CysC is subject to other non-GFR determinants, such as glucocorticoid exposure and thyroid function (50, 52). Contrary to previous suggestions, inflammation does not affect CysC levels (50). In children, CysC has been shown to be a more accurate marker than SCr in specific populations in which muscle mass is diminished, such as children with neural tube defects (53) or in children with a higher muscle mass when mild kidney impairment is suspected (54). Combining both SCr and CysC in the same eGFR equation can lead to better accuracy and precision in estimating GFR (55, 56). That is, the equation becomes less biased and P30-values (P30 is the percentage of patients with eGFR within

30% of mGFR) typically increase with about 5-10%. However, it must be emphasized that averaging the single biomarker SCr-based and CysC-based eGFR equations even leads to better results than using complex composite SCr/CysC-based equations (57). Since body proportions are highly influenced in the context of obesity, CysC might provide an alternative for SCr. While in some studies, CysC-based GFR-estimating equations outperform SCr-based equations in individuals with overweight and obesity (10, 16, 30), this is not the case in other studies (9, 11, 21). Moreover, in individuals with obesity, CysC synthesis has been shown to be upregulated in adipose tissue (58, 59).

Glomerular filtration rate (GFR)-estimating equations and SCr/Q

Glomerular filtration rate (GFR)-estimating equations are developed using the correlation between mGFR and the inverse of endogenous marker concentrations, anthropometric data and other covariates that can influence marker concentrations, such as sex, age and height (60). Correction for these confounders reduces the risk of inaccuracy (bias) and imprecision (random error). Since possibly not all confounding factors are known, an eGFR equation thus reflects the population used for its generation which should be considered when using a specific equation for a specific population and/or individual. Because of this, in **Chapter 2**, the most commonly used SCr-based GFR-estimating equations in children were compared. Serum creatinine (SCr) values of children with overweight and obesity are mostly within the reference range for age and sex for corresponding healthy children and it is known that mGFR is stable between 2 and 40 years of age. Combining these two facts, it is expected to see a flat and sex-independent GFR-age relationship in children with overweight and obesity. In **Chapter 2**, histograms and plots of eGFR versus age including linear quantile regression lines for percentiles 2.5, 50 and 97.5 are presented for the SCr-based GFR-estimating equations. In Table 8.1, an evaluation is presented for the performances of the different SCr-based GFR-estimating equations. Excluded from Table 8.1 is the iCARE equation that was developed in children with overweight and obesity and type 2 diabetes mellitus (9). Because of the BSA-correction in this equation, eGFR iCARE cannot be used in children 12 years old and younger, but seems applicable for adolescents with overweight and obesity. It should be mentioned that iCARE predicts very different values for adolescent males and females (a difference of 15 mL/min/1.73 m²) and 25–30% higher values compared to the other equations.

Table 8.1. Evaluation of different creatinine-based GFR-estimating equations in 600 children with overweight and obesity

	Age/height based	(Relatively) symmetrical distribution?	Age-dependency?	Mean SCr/Q or eGFR differences between males and females ¹	Higher eGFR in children with (severe) obesity? ²
SCr/Qage	age	yes	no	0.04	no
SCr/Qheight	height	yes	no	0.04	yes
eGFR (ml/min/1.73m ²)					
FAS-age (61)	age	yes	no	5.2	no
FAS-height (62)	height	yes	no	5.2	yes
EKFC (63)	age	no	no	5.4	no
CKiD (64)	height	yes	yes	2.8	yes
Schwartz-Lyon (65)	height	yes	yes	7.1	yes
CKiDU25 (66)	height	yes	no	12.4	yes
LMR18 (67)	age	yes	no	6.5	no
CKD-EPI40 (68)	age	no	no	8.1	no

¹ Lower SCr/Q values and higher SCr-based eGFR values in males versus females; we refer to the Supplementary information of Chapter 2 for a detailed evaluation. ² A significant higher eGFR in children with obesity and severe obesity compared to children with overweight. Abbreviations: SCr/Qage, serum creatinine normalized using Q-age polynomials; SCr/Qheight, serum creatinine normalized using Q-height polynomials; eGFR, estimated glomerular filtration rate; FAS, Full-Age Spectrum; EKFC, European Kidney Function Consortium; CKiD, Chronic Kidney Disease in Children; CKiDU25, CKiD under 25 years, LMR18, revised Lund-Malmö extended to children; CKD-EPI40, Chronic Kidney Disease Epidemiology Collaboration extended to children.

As described in **Chapter 2**, considering the distribution of eGFR (which is expected to be relatively symmetrical), absence of age-dependency and order of magnitude of sex-dependency, we suggest that eGFR FAS-age, FAS-height and LMR18 can be allotted as the preferred eGFR equations in children with overweight and obesity aged 2 years and older.

Moreover, as described in **Chapter 3**, we suggest that rescaled SCr (either using Q-age or Q-height polynomials) can be a valuable add-on next to SCr-based GFR-estimating equations. Whilst GFR is indispensable for e.g. the current CKD classification and medication dose adjustment in case of a reduced kidney function, SCr/Q has some advantages compared to eGFR-equations. First, there is no influence of statistical modelling to convert SCr to an eGFR formula. Moreover, there is a clear target value (namely SCr/Q = 1) for a healthy child, independent from age and sex, and a clear reference interval of [0.67 - 1.33]. Finally, in contrary to eGFR (since all eGFR equations are designed for GFR indexed with BSA (7)) repeated measures of SCr/Q over time, are

probably not influenced by BSA which is of major importance since BSA adjustment in individuals with obesity has several disadvantages (18, 20, 69–72).

Recommendation for clinical practice and future research

In conclusion, we come to the following recommendation for GFR determination in children with overweight and obesity. In our opinion, supported by the results from **Chapter 2**, in most cases in clinical setting, SCr can sufficiently reliably be used to estimate GFR in children with overweight and obesity and eGFR FAS-age, FAS-height and LMR18 are the preferred equations. In case of doubt, CysC-based eGFR can be added, in order to combine SCr- and CysC eGFR results. When critical decisions have to be made regarding for instance potentially nephrotoxic drugs or drugs with a narrow therapeutic window, especially in the absence of therapeutic drug monitoring, mGFR should be promoted due to acknowledged inaccuracy of both SCr- and CysC-based eGFR and BSA indexation in individuals with overweight and obesity (28, 73).

Future research should focus on further development of methods for estimating and measuring GFR in children with overweight and obesity. At this moment, the iCARE equation is the only eGFR equation that was developed in children aged 12 years and onwards with obesity and type 2 diabetes mellitus (9). Though, as described in **Chapter 2**, eGFR iCARE predicts very different values for adolescent males and females (a difference of 15 mL/min/1.73 m²) and 25–30% higher values compared to the other equations; these large differences require further investigation and highlight the need for mGFR. Recently, artificial intelligence (AI) and machine learning models have been considered as a new method for GFR estimation (74, 75). We suggest that future research will focus on AI and machine learning models for children in the hope that accuracy and precision of GFR estimation can be improved in children with overweight and obesity.

THE RELATION BETWEEN eGFR AND OBESITY-RELATED COMORBIDITIES IN CHILDHOOD

Obesity is harmful for the kidneys in multiple ways and this detrimental effect of obesity occurs not in the least via obesity-related comorbidities such as hypertension and glucose dysregulation (3). In the general adult population, obesity predicts the onset of CKD (76). The question whether childhood obesity already is related to or leads to kidney damage, cannot easily be answered (77). In **Chapter 1**, we already described the evidence from epidemiological studies on the association of childhood obesity and CKD later in life. In 2019, Jadresic et al. (78) reviewed the evidence for an association between obesity during childhood and kidney function markers. The included cross-sectional population-based studies found a mix of positive, negative and no associations for eGFR and BMI. In 10 included studies a Schwartz equation (most commonly the CKiD 'bedside

Schwartz equation') was used for GFR estimation, and in 9 of these studies, a Schwartz equation was the only equation used. However, given the age-related decline of the CKiD equation in both healthy children (79) and in children with overweight and obesity (described in **Chapter 2**), the CKiD equation might be inappropriate to examine the association between GFR and childhood obesity. Besides, all included studies used BMI as a marker for adiposity, whereas, body composition would be more of interest. In **Chapter 3** we described correlations between eGFR and SCr/Q, and anthropometrical and metabolic variables in 600 children with overweight and obesity without overt kidney disease. Multiple SCr-based eGFR equations and air displacement plethysmography were evaluated. Air displacement plethysmography was only performed in about 48% of the included children, which is a limitation. While SCr/Q-height and some eGFR equations correlated significantly but very weakly with BMI z-score (with univariate correlation coefficients of about 0.10), correlations between eGFR and (the inverse of) SCr/Q versus fat mass and waist-to-hip ratio were more pronounced but still weak (with univariate correlation coefficients of about 0.20-0.30). Moreover, nearly all eGFR-equations and (the inverse of) SCr/Q correlated significantly but very weakly with HOMA-IR and HDL-cholesterol, triacylglyceride, serum uric acid and ALT concentrations (with univariate correlation coefficients of about 0.10). Since this study lacks mGFR, CysC measurements and/or information on enhanced tubular secretion of creatinine, findings have to be interpreted with care. However, our findings support the role of disturbed body composition and other components of the metabolic syndrome (such as hypertension and glucose dysregulation) in the pathogenesis of obesity-related kidney disease (as discussed in **Chapter 1**) (80-82). Besides, it is clear that the choice of a SCr-based eGFR-equation has an enormous impact on possible correlations with anthropometric variables and metabolic risk factors which might explain the inconsistent findings as described in the review by Jadresic et al. (78).

Hypertension and albuminuria

As is the case for all (hyperfiltrative) kidney diseases, screening for and treatment of hypertension and albuminuria is the cornerstone of nephron-protection and thus delaying CKD progression regardless of etiology (83, 84). Screening for hypertension is advised in all current childhood obesity guidelines (85, 86), screening for albuminuria is not. In our cohort, the prevalence of (micro)albuminuria was 6.7% (**Chapter 3**) which is comparable with other studies conducted among children with overweight and obesity (78). Interestingly, this prevalence does not differ substantially from the prevalence of (micro)albuminuria reported in children with normal BMI, which ranges from 3.3% to 12.8% in similar age groups but from across different geographical regions (87, 88). The lack of a higher prevalence of (micro)albuminuria in children with overweight and obesity is striking and not fully understood. It is not excluded that methodological issues in the determination of albuminuria or false-positive results caused by orthostatic proteinuria are involved. Furthermore, since albuminuria in healthy children has

been shown to be negatively associated with BMI, it has been suggested that physical activity may act as a confounding factor (87). Herein we acknowledge the limitation of albuminuria determination within COACH: a random urine spot was used instead of a first morning spot or 24 hour albumin excretion. Still, a random urine spot would expect to result in a false-increased albuminuria determination (due to bias caused by orthostatic proteinuria), and thus albuminuria prevalence is not expected to be false-decreased within our studies.

Hypertension is a, if not the most, common comorbidity in children with overweight and obesity (89-91) and hypertension is a well-known risk factor for the development of CKD (92). Therefore, blood pressure (BP) was evaluated in our cohort of children, described in **Chapter 4**. Within the studies performed using data from participants of the COACH, BP classification was based on a single office-based clinical visit (median 21 measurements per child). This is not consistent with current European Society of Hypertension (ESH) (93) or American Academy of Pediatrics (AAP) (94) hypertension guidelines and therefore is a major limitation. Both guidelines endorse BP classification based on ≥ 3 separate measurement occasions and recommend performing ambulant BP measurement (ABPM) to confirm hypertension. In the clinical setting within the COACH, children with office hypertension underwent office BP follow-up and, if indicated, ABPM.

The absence of ABPM in this thesis has several other limitations as well. First, without ABPM, masked hypertension cannot be identified, which is common in children with overweight and obesity (95) and which is an independent risk factor for end-organ hypertensive damage (96). Moreover, without ABPM there is no information regarding nocturnal hypertension, non-dipping patterns and specifically, the lack of nocturnal BP measurements makes it impossible to examine inference with breathing patterns and ventilation in sleep. This would have been relevant since sleep-disordered breathing (SDB), which is a spectrum of symptoms ranging from primary snoring to obstructive sleep apnea and central sleep apnea, is highly prevalent in children with overweight and obesity and SDB is associated with hypertension and CKD (97-99). The mechanism by which SDB leads to hypertension and CKD includes intermittent nocturnal hypoxia, activation of the sympathetic nervous system and renin angiotensin system, systemic and intra-glomerular hypertension, glomerular hyperfiltration, inflammation and oxidative stress (100). A subgroup of children within the COACH underwent a full polysomnography and some of these data were used in this thesis (**Chapter 3**).

Needless to say, the results of our BP analyses in **Chapter 4**, have to be seen in light of these major limitations and therefore the term 'hypertension' was replaced with the term 'hypertensive BP measurement' in this part of the thesis.

Though, our study described in **Chapter 4** has some strong features and therefore results might contribute to the knowledge of high BP in childhood obesity. The cohort was rather large (554 children with overweight and obesity) and a median of 21 (IQR 20-23) BP measures is a fair percentage of what would be the result of ABPM (ABPM provides on average about 3-4 times as many measurements), with the limitation of the office measurement instead of in free-living conditions. As described in **Chapter 4**, less than half of the children had a normal BP measurement, and 24%, 25% and 6.0% of the children had an elevated BP measurement, stage 1 and stage 2 hypertensive measurement, respectively. Whilst other studies (101-104) do show a positive correlation between BP and BMI z-score in children with obesity, in our study there was only a tendency of this positive correlation; BMI z-score did not differ significantly between the BP categories. Some weak (correlation coefficients of about 0.10) but significant positive correlations were demonstrated between eGFR and both systolic and diastolic BP percentile. In agreement with our results, several studies highlight the association between BP and eGFR in children with overweight and obesity (105-108).

Glucose dysregulation

Besides hypertension, glucose dysregulation is a major well-known risk factor for the development of CKD (92, 109). Insulin resistance and hyperglycemia, both common in children with overweight and obesity (110, 111), are important risk factors for obesity- and diabetes-related kidney disease (80, 112, 113). In **Chapter 5**, we described the glycemic profile in both clinical and free-living conditions of 106 children with overweight and obesity without diabetes and without overt kidney disease, and correlated these findings with eGFR. In the studied children, different stages of glucose dysregulation were already highly present: 56.6% of the children had a HOMA-IR > 2.5 (suggesting insulin resistance), and 23.6% and 3.8% of the children had prediabetes and impaired glucose tolerance, respectively. During 48-hour real-time continuous glucose monitoring (rtCGM), hyperglycemic sensor glucose excursions were present in 26.4% of the children with overweight and obesity. Compared to children without sensor glucose values ≥ 7.8 mmol/L, children with hyperglycemic glucose excursions had a significantly higher average sensor glucose during rtCGM; remarkably no other anthropometric or metabolic variables did significantly differ between the groups. The main finding of this study is that, in children with overweight and obesity with suspected insulin resistance and hyperglycemic glucose excursions, time spent in hyperglycemic range in free living conditions is strongly positively correlated with SCr-based eGFR and the inverse of SCr/Q. These findings support the hypothesis of hyperglycemia-induced hyperfiltration in children with overweight and obesity. To the best of our knowledge, no other studies exist on the correlations between eGFR and elevated glucose levels in free living conditions (using rtCGM) in children with overweight and obesity without diabetes. In (young) adults, multiple studies demonstrated the correlation between prediabetes

glucose dysregulation and estimated (or measured) GFR (and/or presence of glomerular hyperfiltration) (114-119).

Since glucose dysregulation is a risk factor for the development of CKD (92, 109), guidelines advocate strict glycemic targets in order to reduce the onset and progression of CKD in the context of diabetes (120). Although there is evidence that glucose dysregulation in the absence of diabetes is modestly associated with an increase in CKD risk (121), current KDIGO guidelines do not focus on prediabetes and its role on incident CKD and CKD progression (27, 120). Whilst the long-term (kidney-related) complications of youth-onset type 2 diabetes are well-established (122), the consequences of prediabetes among children with overweight and obesity and CKD risk are less known. How best to assess and treat glucose dysregulation including prediabetes and type 2 diabetes mellitus in children is beyond the scope of this thesis, and for this we refer to, for example, the article of Bacha et al. (123) and references therein.

Recommendation for clinical practice and future research

The question whether childhood obesity is already related to or leads to future kidney disease can obviously not be answered in this thesis. What can be said, is that weak correlations between components of the metabolic syndrome and SCr-based eGFR can be found, and that the eGFR equation of choice has an impact on the correlations found (**Chapter 3**). Moreover, we confirmed the high prevalence of hypertensive BP measurements in children with overweight and obesity (**Chapter 4**), underlining the importance of adequate BP measurements in these children, for which we refer to the appropriate guideline (94). Further, in line with other studies conducted among children with overweight and obesity (78), the prevalence of microalbuminuria is low in our cohort of children with overweight and obesity and, in fact, not very different from that in the healthy population (**Chapter 3**) (87, 88). Screening for albuminuria in children with overweight and obesity is not included in current childhood obesity guidelines (85, 86) and based on our thesis we have no arguments to decide otherwise. Finally, we confirmed the high prevalence of glucose dysregulation in children with overweight and obesity without diabetes and found evidence that supported the hypothesis of hyperglycemia-induced glomerular hyperfiltration in this population (**Chapter 5**).

Although the determination of eGFR, hypertension and albuminuria is widely used for the non-invasive assessment of CKD, functional and structural alterations in the kidneys may be present before the detection of these classic markers of kidney damage. In order to detect injury in these early stages, novel biomarkers are researched. Most promising for diagnosis of early kidney tubular and glomerular injury are urinary kidney injury molecule-1 (KIM-1), urinary cystatin C, urinary N-acetyl-beta-D-glucosaminidase (NAG), urinary neutrophil gelatinase-associated lipocalin (NGAL), urinary podocin, urinary nephrin, urinary podocalyxin (PCX), urinary glutamyl aminopeptidase (GluAp), alanyl

aminopeptidase (AlaAp), Klotho, urinary osteopontin and netrin-1 (124, 125). In a group of 142 children with obesity and normal and decreased eGFR, increased urinary concentrations of NGAL have been demonstrated to predict CKD development (126). Further research is needed to confirm the practical usefulness and reliability in the early assessment of obesity-related kidney damage.

Besides this, ectopic lipid accumulation in the kidney ('fatty kidney') can also be considered as a novel biomarker of kidney injury in patients with overweight and obesity, but as for the biomarkers mentioned before, the role in clinical practice is still uncertain. At this moment fatty kidneys can be evaluated with ultrasonography and ultrasound elastography, computed tomography (CT), and magnetic resonance imaging (MRI) (124, 125, 127, 128).

TREATMENT OF OBESITY-RELATED KIDNEY DAMAGE IN CHILDHOOD

Lifestyle intervention

Childhood obesity results from a multifactorial set of socioecological, environmental and genetic influences that act on children and their families (85). Therefore, initial treatment should be lifestyle modification that needs to be child-focused and family-centered with a focus on understanding the underlying genetic, biologic, environmental, and social determinants that are contributing (85). Whilst lifestyle improvement is beneficial in general for individuals with overweight and obesity, it plays a crucial role in primary prevention of CKD as well (129, 130). In a meta-analysis including more than 630,000 adults with no pre-existing kidney disease (131), adherence to a healthy diet was associated with 23% lower odds of incident albuminuria and 30% lower odds of incident CKD. In children with non-glomerular CKD, targeting obesity might improve kidney function (132).

As described in **Chapter 1**, several studies among adults demonstrate that obesity-related kidney disease and/or glomerular hyperfiltration might be reversible in case of weight loss (16, 133-138). In many of these studies, the effect of intervention on GFR, however, might be biased by effects on BSA (as described above). Studies on the effect of weight loss on kidney function in children with overweight and obesity are scarce. To the best of our knowledge, apart from the longitudinal studies described in **Chapters 6 and 7** in this thesis, there are no studies focusing on the effects of lifestyle intervention and eGFR in children with overweight and obesity without overt kidney disease. In **Chapters 6 and 7** we described the effect of the multidisciplinary lifestyle intervention program, that was developed within the COACH, on children with overweight and obesity. It is important to consider that there was a considerable loss to follow-up of children during

the lifestyle intervention (as described in **Chapters 6 and 7**). Whilst loss to follow-up is probably inevitable during lifestyle intervention programs, results might be hampered by the high rate of attrition of the study participants.

In **Chapter 6**, it was demonstrated that one year of lifestyle intervention resulted in a significant improvement of BMI z-score, waist-to-hip ratio, diastolic BP, HbA1c concentration and dyslipidemia. Both SCr and SCr/Q increased and eGFR (unit: ml/min/1.73m²) decreased significantly after one year of lifestyle intervention. De-indexation of eGFR using actual BSA (unit: ml/min) negated this effect. In a subgroup of children with estimated glomerular hyperfiltration (which dramatically reduced the number of children to 32 when a cut-off of eGFR 135 ml/min/1.73m² was used), de-indexed eGFR decreased significantly after one year of lifestyle intervention. No associations of changes in eGFR with changes in BMI z-score, BP or parameters of glucose and lipid metabolism were found.

The study described in **Chapter 6** was conducted prior to the studies in **Chapters 2 and 3** and utilized eGFR FAS-age and CKiD ('bedside Schwartz'). In **Chapter 2**, we propose that eGFR FAS-age, FAS-height, and LMR18 are the preferred equations for estimating GFR in children with overweight and obesity. In **Chapter 3**, we suggest that SCr/Q might serve as an alternative biomarker for evaluating correlations between obesity-related comorbidities and kidney function. When applying the findings from **Chapters 2 and 3** to the earlier dataset from **Chapter 6**, the conclusions remained consistent. Specifically, de-indexed FAS-age, de-indexed FAS-height (and the inverses of both SCr/Q-age and SCr/Q-height), as well as de-indexed LMR18, all showed significant decreases after one year of lifestyle intervention in children with estimated glomerular hyperfiltration.

In order to further evaluate the effect of the lifestyle intervention on eGFR, the study described in **Chapter 7** was performed. In **Chapter 7**, the course of SCr and SCr/Q during 5 years of the COACH lifestyle intervention was described. A yearly SCr/Q increase of about 0.01-0.04 was demonstrated, which equals an eGFR-FAS reduction of about 1.1–4.1 ml/min/1.73m². During lifestyle intervention BMI z-score decreased in both females and males and this reduction was significantly higher in males. There was no correlation between change in BMI z-score and change in SCr/Q. Moreover, there was no difference in change in SCr/Q during follow-up between children that achieved a significant reduction in BMI z-score (the 'cases') and children who did not (the 'controls'). In **Chapter 7**, we already described our hypothesis how the SCr/Q increase and thus eGFR decrease (as described in **Chapters 6 and 7**), during the lifestyle intervention, can possibly be explained. In short:

First, in children with a mean age of about 12.2 ± 3.3 years, the increase in SCr itself is not surprising during lifestyle intervention (and thus with increasing age). The opposite

is true for SCr/Q , which is supposed to be independent of age and sex (Q-age) or height (Q-height). So, increasing age does not explain increasing SCr/Q during lifestyle intervention.

Second, it seems plausible that during a lifestyle intervention program, the amount of muscle mass not only increases with age, but also resulting from more physical activity. Increase in muscle mass leads to higher SCr levels and, since this effect is age- and height-independent, also increased SCr/Q . In **Chapter 7**, when fat mass was added as an extra covariate (which seriously reduces the number of observations used, a limitation that has already been described), a significant effect on change in SCr/Q could not be demonstrated. So, the SCr/Q increase during the lifestyle intervention could not be explained by an increase in muscle mass in our study, though the results might be hampered by missing data on fat mass.

Third, there is evidence of enhanced tubular secretion of creatinine in individuals with overweight and obesity (39, 42). From both animal and clinical studies, it is demonstrated that tubular injury can be reversible following weight loss. A study in dogs demonstrated reversibility of both glomerular and tubular injury, the latter determined using urinary immunoglobulin G and urinary NGAL (139). In 22 adolescents with severe obesity, baseline levels of urinary NGAL, interleukin-8 (IL-8), and KIM-1 were elevated compared to lean controls, but these levels did not significantly change following weight loss induced by bariatric surgery (140). The impact of lifestyle intervention on tubular secretion of creatinine in children with overweight and obesity requires further research.

Finally, there is the hyperfiltration hypothesis. Multiple studies suggested that obesity-related hyperfiltration might be reversible in case of weight loss (16, 133-138). So, one could expect that during lifestyle intervention, this is also the case in children in whom obesity-related hyperfiltration might already be present. In **Chapter 6**, we indeed demonstrated a clear and significant decrease in de-indexed $eGFR$ in children with presumed glomerular hyperfiltration at baseline. Change in BMI z-score was not correlated with change in $eGFR$ and SCr/Q in both **Chapters 6 and 7**. Moreover, in **Chapter 7**, we could not demonstrate a difference in change in SCr/Q during follow-up between children that achieved a significant reduction in BMI z-score (the 'cases') and children who did not (the 'controls').

In conclusion, both **Chapters 6 and 7** demonstrates that SCr/Q slightly increases (and thus $eGFR$ slightly decreases) during lifestyle intervention in children with overweight and obesity. Whether this minor change in $eGFR$ is a favorable outcome and has clinical consequences on the long-term, remains to be determined in trials with a longer period of follow-up. To the best of our knowledge, no other studies to date have evaluated $eGFR$

during the course of a lifestyle intervention program in children with overweight and obesity.

Other treatment options

Treatment of severe childhood obesity extends beyond lifestyle modification (85). Whilst in this thesis we did not investigate other treatment options beyond lifestyle intervention in children with overweight and obesity, evidence is accumulating for the positive contributions of these other therapies on kidney health. Here, we briefly describe the results of both metabolic bariatric surgery (MBS) and pharmacotherapeutic agents on kidney outcomes.

A study among 242 adolescents demonstrated significant improvement in weight, cardiometabolic health, weight-related quality of life, and also a significant reduction of albuminuria and an increase in eGFR 3 and 5 years after MBS (141-143). Note that GFR was estimated using the FAS combined SCr and CysC equation (144). Estimated GFR was indexed for BSA and not de-indexed for BSA after intervention and so, the effect of MBS on eGFR therefore might rather be a result from changes in BSA rather than an effect on absolute GFR, which is, in fact, a common concern in kidney function evaluation after MBS (145). Moreover, after MBS, creatinine production decreases by about 20–25%, due to the loss of not only fat mass, but also muscle mass, resulting in a decrease in SCr levels (146) and thus higher SCr-based GFR estimates. Despite these concerns, literature in adults suggests that in patients with hyperfiltration at baseline, GFR is reduced following MBS, whereas GFR is increased in patients with decreased GFR at baseline (147-149). These findings are also confirmed in the few studies that have used mGFR rather than eGFR (133, 134, 150, 151). Besides improvements in GFR and albuminuria, MBS has been demonstrated to improve urinary excretion of sphingolipids in adolescents with severe obesity (152). So, MBS might be considered as a kidney-protective treatment option in adolescents with severe obesity (153).

In addition to lifestyle intervention and MBS, pharmacological treatments are increasingly being used for the management of obesity. The two major classes of drugs are glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RA) and sodium glucose co-transporter 2 (SGLT2) inhibitors (SGLT2i) (125).

Multiple studies demonstrated beneficial outcomes of GLP-1RA liraglutide, semaglutide and dulaglutide regarding weight loss and ameliorating kidney function in adults with type 2 diabetes mellitus (154-157). In adults without diabetes and with overweight and obesity, treatment with liraglutide and semaglutide has shown beneficial effects regarding weight loss (158, 159). Moreover, treatment with semaglutide improved cardiovascular outcome and resulted in reduction of albuminuria in adults with overweight and obesity without diabetes (160, 161). So, there is strong evidence that GLP-1RA reduce clinically

important kidney events, kidney failure and cardiovascular events in adults (162). Recently, two GLP-1RA (liraglutide and semaglutide) have received FDA approval for the treatment of obesity in adolescents ≥ 12 years (163, 164). There is increasing evidence that GLP-1RA, in particular semaglutide, demonstrate favorable outcomes in children and adolescents with overweight and obesity without diabetes (165). More specifically, in 229 adolescents with obesity (only 4% had diabetes), semaglutide plus lifestyle intervention resulted in a greater reduction in BMI compared with lifestyle intervention alone; the mean change in BMI from baseline to week 68 was -16.1% with semaglutide and 0.6% with placebo (estimated difference, -16.7 percentage points; 95% CI, -20.3 to -13.2; $p < 0.001$) (164). Finally, in 82 children (aged 6 to <12 years), liraglutide for 56 weeks plus lifestyle interventions resulted in a greater reduction in BMI than placebo plus lifestyle interventions; the mean change in BMI from baseline to week 56 was -5.8% with liraglutide and 1.6% with placebo (estimated difference, -7.4 percentage points; 95% CI, -11.6 to -3.2; $p < 0.001$) (166).

In contrast to GLP-1RA, SGLT2i have not gained FDA approval for the treatment of obesity in the absence of diabetes. However, both dapagliflozin (167, 168) and empagliflozin (169) received FDA approval for youth-onset type 2 diabetes. Neither one of these agents had a significant effect on BMI reduction in children. In adults with diabetes, SGLT2i were associated with significant reduction in HbA1c and body weight (170) and decrease the risk of major cardiovascular events, heart failure, and kidney failure (171, 172). In adults, several trials have demonstrated modest weight reductions and positive effects on kidney health following treatment with SGLT2i independent of diabetes status (173, 174). Whilst there are short-term follow-up studies on SGLT2i and CKD progression in children (175, 176), data on this are still limited and results from ongoing and future trials are to be awaited (177).

Recommendations for clinical practice and future research

The cornerstone in the treatment of childhood obesity is lifestyle modification (85, 86). As described, lifestyle modification plays a crucial role in the primary prevention of CKD as well (129, 130). In this thesis (**Chapters 6 and 7**) we demonstrated that eGFR slightly decreases during lifestyle intervention in children with overweight and obesity. Whether this minor change in eGFR has clinical consequences on the long-term, remains to be determined in trials with a longer period of follow-up. In addition to lifestyle modification, MBS and pharmacological agents like GLP-1RA and SGLT2i can be considered for the treatment of severe obesity, with potential benefits for kidney function. Future research is needed to better understand the potential nephron-protective effect of lifestyle intervention, weight loss and/or pharmacotherapies. All kidney-protective drugs (like RAAS inhibitors, SGLT2i and GLP-1RA) in clinical use so far induce an early decrease in GFR, which is thought to result from reversal of glomerular hyperfiltration (178). Despite this and the evidence that glomerular hyperfiltration contributes to CKD progression,

it is still unclear whether individuals with glomerular hyperfiltration per se (so without albuminuria, proteinuria, or hypertension) should be treated (178, 179). There is a clear need for studies evaluating potential clinical indicators for glomerular hyperfiltration to establish this phenomenon as a valid therapeutic target (178).

CLOSING REMARKS

Current evidence suggests that excess weight in children puts the kidneys under pressure, even though the exact mechanisms linking childhood obesity to kidney dysfunction are not yet fully understood. In this thesis, the majority of children with overweight and obesity had a normal SCr-based eGFR. However, associations were found between adiposity and both high blood pressure and impaired glucose regulation, two factors known to adversely affect kidney health. Additionally, previous research has demonstrated that obesity is a negative prognostic factor in children with pre-existing kidney disease. Other studies have also shown that childhood obesity increases the risk of developing CKD later in life. Awareness is growing that obesity should be recognized as a modifiable risk factor in the development and progression of CKD. Ultimately, early prevention and targeted follow-up of children with overweight and obesity could help reduce the risk of kidney disease progression into adulthood.

REFERENCES

1. Kovesdy CP, Furth SL, Zoccali C. Obesity and kidney disease: hidden consequences of the epidemic. *Pediatr Nephrol*. 2017;32(4):537-45
2. Time to sound the alarm about the hidden epidemic of kidney disease. *Nature*. 2024;628(8006):7-8
3. Nehus E, Mitsnefes M. Kidney consequences of obesity. *Pediatr Nephrol*. 2025 Jun;40(6):1879-1893
4. Piepsz A, Tondeur M, Ham H. Revisiting normal (51)Cr-ethylenediaminetetraacetic acid clearance values in children. *Eur J Nucl Med Mol Imaging*. 2006;33(12):1477-82
5. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. *Clin J Am Soc Nephrol*. 2009;4(11):1832-43
6. Smeets NJL, Int'Hout J, van der Burgh MJP, Schwartz GJ, Schreuder MF, de Wildt SN. Maturation of GFR in Term-Born Neonates: An Individual Participant Data Meta-Analysis. *J Am Soc Nephrol*. 2022;33(7):1277-92
7. Pottel H. Measuring and estimating glomerular filtration rate in children. *Pediatr Nephrol*. 2017;32(2):249-63
8. Correia-Costa L, Schaefer F, Afonso AC, Bustorff M, Guimarães JT, Guerra A, et al. Normalization of glomerular filtration rate in obese children. *Pediatr Nephrol*. 2016;31(8):1321-8
9. Dart AB, McGavock J, Sharma A, Chateau D, Schwartz GJ, Blydt-Hansen T. Estimating glomerular filtration rate in youth with obesity and type 2 diabetes: the iCARE study equation. *Pediatr Nephrol*. 2019;34(9):1565-74
10. Lemoine S, Panaye M, Pelletier C, Bon C, Juillard L, Dubourg L, et al. Cystatin C-Creatinine Based Glomerular Filtration Rate Equation in Obese Chronic Kidney Disease Patients: Impact of Deindexation and Gender. *Am J Nephrol*. 2016;44(1):63-70
11. Friedman AN, Strother M, Quinney SK, Hall S, Perkins SM, Brizendine EJ, et al. Measuring the glomerular filtration rate in obese individuals without overt kidney disease. *Nephron Clin Pract*. 2010;116(3):c224-34
12. Ozmen S, Kaplan MA, Kaya H, Akin D, Danis R, Kizilkan B, et al. Role of lean body mass for estimation of glomerular filtration rate in patients with chronic kidney disease with various body mass indices. *Scand J Urol Nephrol*. 2009;43(2):171-6
13. Verhave JC, Fesler P, Ribstein J, du Cailar G, Mimran A. Estimation of renal function in subjects with normal serum creatinine levels: influence of age and body mass index. *Am J Kidney Dis*. 2005;46(2):233-41
14. Bouqueneau A, Vidal-Petiot E, Moranne O, Mariat C, Boffa JJ, Vrtovnik F, et al. Creatinine-based equations for the adjustment of drug dosage in an obese population. *Br J Clin Pharmacol*. 2016;81(2):349-61
15. Lemoine S, Guebre-Egziabher F, Sens F, Nguyen-Tu MS, Juillard L, Dubourg L, et al. Accuracy of GFR estimation in obese patients. *Clin J Am Soc Nephrol*. 2014;9(4):720-7
16. von Scholten BJ, Persson F, Svane MS, Hansen TW, Madsbad S, Rossing P. Effect of large weight reductions on measured and estimated kidney function. *BMC Nephrol*. 2017;18(1):52
17. Redal-Baigorry B, Rasmussen K, Heaf JG. The use of absolute values improves performance of estimation formulae: a retrospective cross sectional study. *BMC Nephrol*. 2013;14:271.
18. Delanaye P, Radermecker RP, Rorive M, Depas G, Krzesinski JM. Indexing glomerular filtration rate for body surface area in obese patients is misleading: concept and example. *Nephrol Dial Transplant*. 2005;20(10):2024-8

19. Delanaye P, Mariat C, Cavalier E, Krzesinski JM. Errors induced by indexing glomerular filtration rate for body surface area: reductio ad absurdum. *Nephrol Dial Transplant*. 2009;24(12):3593-6
20. Delanaye P, Krzesinski JM. Indexing of renal function parameters by body surface area: intelligence or folly? *Nephron Clin Pract*. 2011;119(4):c289-92
21. López-Martínez M, Luis-Lima S, Morales E, Navarro-Díaz M, Negrín-Mena N, Folgueras T, et al. The estimation of GFR and the adjustment for BSA in overweight and obesity: a dreadful combination of two errors. *Int J Obes (Lond)*. 2020;44(5):1129-40
22. Ebert N, Schaeffner E, Seegmiller JC, van Londen M, Bökenkamp A, Cavalier E, et al. Iohexol plasma clearance measurement protocol standardization for adults: a consensus paper of the European Kidney Function Consortium. *Kidney Int*. 2024;106(4):583-96
23. Delanaye P, Ebert N, Melsom T, Gaspari F, Mariat C, Cavalier E, et al. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: How to measure glomerular filtration rate with iohexol? *Clin Kidney J*. 2016;9(5):682-99
24. Staples A, Wong C, Schwartz GJ. Iohexol-measured glomerular filtration rate in children and adolescents with chronic kidney disease: a pilot study comparing venous and finger stick methods. *Pediatr Nephrol*. 2019;34(3):459-64
25. den Bakker E, Gemke R, Bökenkamp A. Endogenous markers for kidney function in children: a review. *Crit Rev Clin Lab Sci*. 2018;55(3):163-83
26. Pottel H, Schwartz GJ. Measuring and estimating the GFR in children: state of the art in 2025. *Pediatr Nephrol*. 2025 Nov;40(11):3347-3357
27. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int*. 2024;105(4s):S117-s314
28. Delanaye P, Pottel H, Cavalier E, Flamant M, Stehlé T, Mariat C. Diagnostic standard: assessing glomerular filtration rate. *Nephrol Dial Transplant*. 2024;39(7):1088-96
29. Bostan Gayret Ö, Taşdemir M, Erol M, Tekin Nacaroğlu H, Zengi O, Yiğit Ö. Are there any new reliable markers to detect renal injury in obese children? *Renal Fail*. 2018;40(1):416-22.
30. Önerli Salman D, Şıklar Z, Çullas İlarslan EN, Özçakar ZB, Kocaay P, Berberoğlu M. Evaluation of Renal Function in Obese Children and Adolescents Using Serum Cystatin C Levels, Estimated Glomerular Filtration Rate Formulae and Proteinuria: Which is most Useful? *J Clin Res Pediatr Endocrinol*. 2019 Feb 20;11(1):46-5
31. Martin-Del-Campo F, Batis-Ruvalcaba C, Ordaz-Medina SM, Martínez-Ramírez HR, Vizmanos-Lamotte B, Romero-Velarde E, et al. Frequency and Risk Factors of Kidney Alterations in Children and Adolescents who Are Overweight and Obese in a Primary Health-care Setting. *J Ren Nutr*. 2019;29(5):370-6
32. Murphy MO, Huang H, Bauer JA, Schadler A, Makhoul M, Clasey JL, et al. Impact of Pediatric Obesity on Diurnal Blood Pressure Assessment and Cardiovascular Risk Markers. *Front Pediatr*. 2021;9:596142
33. Morato M, Correia-Costa L, Sousa T, Cosme D, Schaefer F, Areias JC, et al. Longer duration of obesity is associated with a reduction in urinary angiotensinogen in prepubertal children. *Pediatr Nephrol*. 2017;32(8):1411-22
34. Di Bonito P, Licenziati MR, Campana G, Chiesa C, Pacifico L, Manco M, et al. Prevalence of Mildly Reduced Estimated GFR by Height- or Age-Related Equations in Young People With Obesity and Its Association with Cardiometabolic Risk Factors. *J Ren Nutr*. 2021;31(6):586-92
35. Correia-Costa L, Afonso AC, Schaefer F, Guimarães JT, Bustorff M, Guerra A, et al. Decreased renal function in overweight and obese prepubertal children. *Pediatr Res*. 2015;78(4):436-44
36. Wells JC, Fewtrell MS, Williams JE, Haroun D, Lawson MS, Cole TJ. Body composition in normal weight, overweight and obese children: matched case-control analyses of total and regional

- tissue masses, and body composition trends in relation to relative weight. *Int J Obes (Lond)*. 2006;30(10):1506-13
37. Eisner C, Faulhaber-Walter R, Wang Y, Leelahavanichkul A, Yuen PS, Mizel D, et al. Major contribution of tubular secretion to creatinine clearance in mice. *Kidney Int*. 2010;77(6):519-26
 38. Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. *Annu Rev Med*. 1988;39:465-90
 39. Sinkeler SJ, Visser FW, Krikken JA, Stegeman CA, Homan van der Heide JJ, Navis G. Higher body mass index is associated with higher fractional creatinine excretion in healthy subjects. *Nephrol Dial Transplant*. 2011;26(10):3181-8
 40. Herrera J, Avila E, Marín C, Rodríguez-Iturbe B. Impaired creatinine secretion after an intravenous creatinine load is an early characteristic of the nephropathy of sickle cell anaemia. *Nephrol Dial Transplant*. 2002;17(4):602-7
 41. Rodríguez-Iturbe B, Herrera J, Marín C, Mañalich R. Tubular stress test detects subclinical reduction in renal functioning mass. *Kidney Int*. 2001;59(3):1094-102
 42. Goumenos DS, Kavar B, El Nahas M, Conti S, Wagner B, Spyropoulos C, et al. Early histological changes in the kidney of people with morbid obesity. *Nephrol Dial Transplant*. 2009;24(12):3732-8
 43. Lu X, Dong Y, Jian Z, Li Q, Gong L, Tang L, et al. Systematic Investigation of the Effects of Long-Term Administration of a High-Fat Diet on Drug Transporters in the Mouse Liver, Kidney and Intestine. *Curr Drug Metab*. 2019;20(9):742-55
 44. Pottel H, Vrydags N, Mahieu B, Vandewynckele E, Croes K, Martens F. Establishing age/sex related serum creatinine reference intervals from hospital laboratory data based on different statistical methods. *Clin Chim Acta*. 2008;396(1-2):49-55
 45. Ceriotti F, Boyd JC, Klein G, Henny J, Queralto J, Kairisto V, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem*. 2008;54(3):559-66
 46. Pottel H, Hoste L, Delanaye P. Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m(2). *Pediatr Nephrol*. 2015;30(5):821-8
 47. Pottel H, Adebayo OC, Nkoy AB, Delanaye P. Glomerular hyperfiltration: part 1 - defining the threshold - is the sky the limit? *Pediatr Nephrol*. 2023 Aug;38(8):2523-2527
 48. Grubb A, Simonsen O, Sturfelt G, Truedsson L, Thysell H. Serum concentration of cystatin C, factor D and beta 2-microglobulin as a measure of glomerular filtration rate. *Acta Med Scand*. 1985;218(5):499-503
 49. Miliku K, Bakker H, Dorresteijn EM, Cransberg K, Franco OH, Felix JF, et al. Childhood Estimates of Glomerular Filtration Rate Based on Creatinine and Cystatin C: Importance of Body Composition. *Am J Nephrol*. 2017;45(4):320-6
 50. Malmgren L, Öberg C, den Bakker E, Leion F, Siódmiak J, Åkesson A, et al. The complexity of kidney disease and diagnosing it - cystatin C, selective glomerular hypofiltration syndromes and proteome regulation. *J Intern Med*. 2023;293(3):293-308
 51. Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Brodehl J. Reference values for cystatin C serum concentrations in children. *Pediatr Nephrol*. 1998;12(2):125-9
 52. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. *Kidney Int*. 2009;75(6):652-60
 53. Pham-Huy A, Leonard M, Lepage N, Halton J, Filler G. Measuring glomerular filtration rate with cystatin C and beta-trace protein in children with spina bifida. *J Urol*. 2003;169(6):2312-5

54. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol*. 2008;3(2):348-54
55. Pottel H, Björk J, Rule AD, Ebert N, Eriksen BO, Dubourg L, et al. Cystatin C-Based Equation to Estimate GFR without the Inclusion of Race and Sex. *N Engl J Med*. 2023;388(4):333-43
56. Fu EL, Levey AS, Coresh J, Elinder CG, Rotmans JJ, Dekker FW, et al. Accuracy of GFR Estimating Equations in Patients with Discordances between Creatinine and Cystatin C-Based Estimations. *J Am Soc Nephrol*. 2023;34(7):1241-51
57. Pottel H, Delanaye P, Cavalier E. Exploring Renal Function Assessment: Creatinine, Cystatin C, and Estimated Glomerular Filtration Rate Focused on the European Kidney Function Consortium Equation. *Ann Lab Med*. 2024;44(2):135-43
58. Dedual MA, Wueest S, Challa TD, Lucchini FC, Aeppli TRJ, Borsigova M, et al. Obesity-Induced Increase in Cystatin C Alleviates Tissue Inflammation. *Diabetes*. 2020;69(9):1927-35
59. Naour N, Fellahi S, Renucci JF, Poitou C, Rouault C, Basdevant A, et al. Potential contribution of adipose tissue to elevated serum cystatin C in human obesity. *Obesity (Silver Spring)*. 2009;17(12):2121-6
60. den Bakker E, Bökenkamp A, Haffner D. Assessment of Kidney Function in Children. *Pediatr Clin North Am*. 2022;69(6):1017-1035
61. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol*. 2012;27(6):973-9
62. Hoste L, Dubourg L, Selistre L, De Souza VC, Ranchin B, Hadj-Aïssa A, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. *Nephrol Dial Transplant*. 2014;29(5):1082-91
63. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, et al. Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate : A Cross-sectional Analysis of Pooled Data. *Ann Intern Med*. 2021;174(2):183-91
64. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20(3):629-37
65. De Souza VC, Rabilloud M, Cochat P, Selistre L, Hadj-Aïssa A, Kassai B, et al. Schwartz formula: is one k-coefficient adequate for all children? *PLoS One*. 2012;7(12):e53439
66. Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ. Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. *Kidney Int*. 2021;99(4):948-56
67. Björk J, Nyman U, Delanaye P, Grubb A, Larsson A, Vranken L, et al. A novel method for creatinine adjustment makes the revised Lund-Malmö GFR estimating equation applicable in children. *Scand J Clin Lab Invest*. 2020;80(6):456-63
68. Björk J, Nyman U, Larsson A, Delanaye P, Pottel H. Estimation of the glomerular filtration rate in children and young adults by means of the CKD-EPI equation with age-adjusted creatinine values. *Kidney Int*. 2021;99(4):940-7
69. Schmieder RE, Beil AH, Weihprecht H, Messerli FH. How should renal hemodynamic data be indexed in obesity? *J Am Soc Nephrol*. 1995;5(9):1709-13
70. Anastasio P, Spitali L, Frangiosa A, Molino D, Stellato D, Cirillo E, et al. Glomerular filtration rate in severely overweight normotensive humans. *Am J Kidney Dis*. 2000;35(6):1144-8
71. Chagnac A, Weinstein T, Korzets A, Ramadan E, Hirsch J, Gaftor U. Glomerular hemodynamics in severe obesity. *Am J Physiol Renal Physiol*. 2000;278(5):F817-22
72. Chang AR, Zafar W, Grams ME. Kidney Function in Obesity-Challenges in Indexing and Estimation. *Adv Chronic Kidney Dis*. 2018;25(1):31-40

73. Ebert N, Bevc S, Bökenkamp A, Gaillard F, Hornum M, Jager KJ, et al. Assessment of kidney function: clinical indications for measured GFR. *Clin Kidney J.* 2021;14(8):1861-70
74. Nakano FK, Åkesson A, de Boer J, Dedja K, D'Hondt R, Haredasht FN, et al. Comparison between the EKFC-equation and machine learning models to predict Glomerular Filtration Rate. *Sci Rep.* 2024;14(1):26383
75. Lanot A, Akesson A, Nakano FK, Vens C, Björk J, Nyman U, et al. Enhancing individual glomerular filtration rate assessment: can we trust the equation? Development and validation of machine learning models to assess the trustworthiness of estimated GFR compared to measured GFR. *BMC Nephrol.* 2025;26(1):47
76. Garofalo C, Borrelli S, Minutolo R, Chiodini P, De Nicola L, Conte G. A systematic review and meta-analysis suggests obesity predicts onset of chronic kidney disease in the general population. *Kidney Int.* 2017;91(5):1224-35
77. Barlabà A, Grella C, Tammaro M, Petrone D, Guarino S, Miraglia Del Giudice E, et al. Kidney function evaluation in children and adolescents with obesity: a not-negligible need. *Eur J Pediatr.* 2024;183(9):3655-64
78. Jadresic L, Silverwood RJ, Kinra S, Nitsch D. Can childhood obesity influence later chronic kidney disease? *Pediatr Nephrol.* 2019;34(12):2457-77
79. Pottel H, Björk J, Delanaye P, Nyman U. Evaluation of the creatinine-based chronic kidney disease in children (under 25 years) equation in healthy children and adolescents. *Pediatr Nephrol.* 2022;37(9):2213-6
80. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol.* 2016;12(8):453-71
81. Sharma I, Liao Y, Zheng X, Kanwar YS. New Pandemic: Obesity and Associated Nephropathy. *Front Med (Lausanne).* 2021 Jun 29;8:673556
82. Lin L, Tan W, Pan X, Tian E, Wu Z, Yang J. Metabolic Syndrome-Related Kidney Injury: A Review and Update. *Front Endocrinol (Lausanne).* 2022;13:904001
83. Wühl E, Schaefer F. Therapeutic strategies to slow chronic kidney disease progression. *Pediatr Nephrol.* 2008;23(5):705-16
84. Atkinson MA, Ng DK, Warady BA, Furth SL, Flynn JT. The CKiD study: overview and summary of findings related to kidney disease progression. *Pediatr Nephrol.* 2021;36(3):527-38
85. Hampl SE, Hassink SG, Skinner AC, Armstrong SC, Barlow SE, Bolling CF, et al. Clinical Practice Guideline for the Evaluation and Treatment of Children and Adolescents With Obesity. *Pediatrics.* 2023;151(2)
86. National Institute for Health and Care Excellence (NICE): Guidelines. Obesity: identification, assessment and management. 2023 Jul 26
87. Gracchi V, van den Belt SM, Corpeleijn E, Heerspink HJL, Verkade HJ. Albuminuria and markers for cardiovascular risk in 12-year-olds from the general Dutch population: a cross-sectional study. *Eur J Pediatr.* 2023;182(11):4921-9
88. Larkins N, Teixeira-Pinto A, Craig J. The population-based prevalence of albuminuria in children. *Pediatr Nephrol.* 2017;32(12):2303-9
89. Litwin M, Kułaga Z. Obesity, metabolic syndrome, and primary hypertension. *Pediatr Nephrol.* 2021;36(4):825-37
90. Wühl E. Hypertension in childhood obesity. *Acta Paediatr.* 2019;108(1):37-43
91. Flechtner-Mors M, Thamm M, Wiegand S, Reinehr T, Schwab KO, Kiess W, et al. Comorbidities related to BMI category in children and adolescents: German/Austrian/Swiss Obesity Register APV compared to the German KiGGS Study. *Horm Res Paediatr.* 2012;77(1):19-26

92. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. *Lancet*. 2017;389(10075):1238-52
93. Mancia G, Kreutz R, Brunström M, Burnier M, Grassi G, Januszewicz A, et al. 2023 ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension: Endorsed by the International Society of Hypertension (ISH) and the European Renal Association (ERA). *J Hypertens*. 2023;41(12):1874-2071
94. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics*. 2017;140(3)
95. Aguilar A, Ostrow V, De Luca F, Suarez E. Elevated ambulatory blood pressure in a multi-ethnic population of obese children and adolescents. *J Pediatr*. 2010;156(6):930-5
96. Stabouli S, Kotsis V, Toumanidis S, Papamichael C, Constantopoulos A, Zakopoulos N. White-coat and masked hypertension in children: association with target-organ damage. *Pediatr Nephrol*. 2005;20(8):1151-5
97. Bradley TD, Floras JS. Obstructive sleep apnoea and its cardiovascular consequences. *Lancet*. 2009;373(9657):82-93
98. Hwu DW, Lin KD, Lin KC, Lee YJ, Chang YH. The association of obstructive sleep apnea and renal outcomes-a systematic review and meta-analysis. *BMC Nephrol*. 2017;18(1):313
99. Rodriguez-Lopez S, Ofosu D, Gerdung C, Keto-Lambert D, Sebastianski M, Lin M, et al. Childhood Obstructive Sleep Apnea and Systemic Blood Pressure and Kidney Function: A Systematic Review and Meta-Analysis. *Int J Hypertens*. 2025;2025:1945725
100. Lin CH, Perger E, Lyons OD. Obstructive sleep apnea and chronic kidney disease. *Curr Opin Pulm Med*. 2018;24(6):549-54
101. Sorof JM, Lai D, Turner J, Poffenbarger T, Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics*. 2004;113(3 Pt 1):475-82
102. Steinhorsdottir SD, Eliasdottir SB, Indridason OS, Agustsdottir IM, Palsson R, Edvardsson VO. Prevalence of hypertension in 9- to 10-year-old Icelandic school children. *J Clin Hypertens (Greenwich)*. 2011;13(10):774-9
103. Tu W, Eckert GJ, DiMeglio LA, Yu Z, Jung J, Pratt JH. Intensified effect of adiposity on blood pressure in overweight and obese children. *Hypertension*. 2011;58(5):818-24
104. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. *Pediatrics*. 1999;103(6 Pt 1):1175-82
105. Xargay-Torrent S, Puerto-Carranza E, Marcelo I, Mas-Parés B, Gómez-Vilarrubla A, Martínez-Calcerrada JM, et al. Estimated glomerular filtration rate and cardiometabolic risk factors in a longitudinal cohort of children. *Sci Rep*. 2021;11(1):11702
106. Koulouridis E, Georgalidis K, Kostimpa I, Koulouridis I, Krokida A, Houliara D. Metabolic syndrome risk factors and estimated glomerular filtration rate among children and adolescents. *Pediatr Nephrol*. 2010;25(3):491-8
107. Ricotti R, Genoni G, Giglione E, Monzani A, Nuges M, Zanetta S, et al. High-normal estimated glomerular filtration rate and hyperuricemia positively correlate with metabolic impairment in pediatric obese patients. *PLoS One*. 2018;13(3):e0193755
108. Kelishadi R, Qorbani M, Assadi F, Motlagh ME, Djalalinia S, Shahsavari A, et al. Glomerular Hyperfiltration as Predictor of Cardiometabolic Risk Factors among Children and Adolescents: The Childhood and Adolescence Surveillance and Prevention of Adult-V Study. *Int J Prev Med*. 2018;9:33

109. Farag YM, Gaballa MR. Diabetes: an overview of a rising epidemic. *Nephrol Dial Transplant*. 2011;26(1):28-35
110. Rijks J, Karnebeek K, van Dijk JW, Dorenbos E, Gerver WJ, Stouthart P, et al. Glycaemic Profiles of Children With Overweight and Obesity in Free-living Conditions in Association With Cardiometabolic Risk. *Sci Rep*. 2016;6:31892
111. Hegedus E, Salvy SJ, Wee CP, Naguib M, Raymond JK, Fox DS, et al. Use of continuous glucose monitoring in obesity research: A scoping review. *Obes Res Clin Pract*. 2021;15(5):431-8
112. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *J Am Soc Nephrol*. 2017;28(4):1023-39
113. Sawyer A, Zeitler E, Trachtman H, Bjornstad P. Kidney Considerations in Pediatric Obesity. *Curr Obes Rep*. 2023;12(3):332-44
114. Tomaszewski M, Charchar FJ, Maric C, McClure J, Crawford L, Grzeszczak W, et al. Glomerular hyperfiltration: a new marker of metabolic risk. *Kidney Int*. 2007;71(8):816-21
115. Pruijm M, Wuerzner G, Maillard M, Bovet P, Renaud C, Bochud M, et al. Glomerular hyperfiltration and increased proximal sodium reabsorption in subjects with type 2 diabetes or impaired fasting glucose in a population of the African region. *Nephrol Dial Transplant*. 2010;25(7):2225-31
116. Melsom T, Mathisen UD, Ingebretsen OC, Jenssen TG, Njolstad I, Solbu MD, et al. Impaired fasting glucose is associated with renal hyperfiltration in the general population. *Diabetes Care*. 2011;34(7):1546-51
117. Okada R, Yasuda Y, Tushita K, Wakai K, Hamajima N, Matsuo S. Glomerular hyperfiltration in prediabetes and prehypertension. *Nephrol Dial Transplant*. 2012;27(5):1821-5
118. Sun ZJ, Yang YC, Wu JS, Wang MC, Chang CJ, Lu FH. Increased risk of glomerular hyperfiltration in subjects with impaired glucose tolerance and newly diagnosed diabetes. *Nephrol Dial Transplant*. 2016;31(8):1295-301
119. Melsom T, Schei J, Stefansson VT, Solbu MD, Jenssen TG, Mathisen UD, et al. Prediabetes and Risk of Glomerular Hyperfiltration and Albuminuria in the General Nondiabetic Population: A Prospective Cohort Study. *Am J Kidney Dis*. 2016;67(6):841-50
120. KDIGO 2022 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. *Kidney Int*. 2022;102(5s):S1-s127
121. Echouffo-Tcheugui JB, Narayan KM, Weisman D, Golden SH, Jaar BG. Association between prediabetes and risk of chronic kidney disease: a systematic review and meta-analysis. *Diabet Med*. 2016;33(12):1615-24
122. Bjornstad P, Drews KL, Caprio S, Gubitosi-Klug R, Nathan DM, Tesfaldet B, et al. Long-Term Complications in Youth-Onset Type 2 Diabetes. *N Engl J Med*. 2021;385(5):416-26
123. Bacha F, Hannon TS, Tosur M, Pike JM, Butler A, Tommerdahl KL, et al. Pathophysiology and Treatment of Prediabetes and Type 2 Diabetes in Youth. *Diabetes Care*. 2024;47(12):2038-49
124. Martínez-Montoro JI, Morales E, Cornejo-Pareja I, Tinahones FJ, Fernández-García JC. Obesity-related glomerulopathy: Current approaches and future perspectives. *Obes Rev*. 2022;23(7):e13450
125. Kounatidis D, Vallianou NG, Stratigou T, Voukali M, Karampela I, Dalamaga M. The Kidney in Obesity: Current Evidence, Perspectives and Controversies. *Curr Obes Rep*. 2024 Dec;13(4):680-702
126. Mackowiak-Lewandowicz K, Ostalska-Nowicka D, Zaorska K, Kaczmarek E, Zachwieja J, Witt M, et al. Chronic kidney disease predictors in obese adolescents. *Pediatr Nephrol*. 2022;37(10):2479-88

127. Jonker JT, de Heer P, Engelse MA, van Rossenberg EH, Klessens CQF, Baelde HJ, et al. Metabolic imaging of fatty kidney in diabetes: validation and dietary intervention. *Nephrol Dial Transplant*. 2018;33(2):224-30
128. Hti Lar Seng NS, Lohana P, Chandra S, Jim B. The Fatty Kidney and Beyond: A Silent Epidemic. *Am J Med*. 2023;136(10):965-74
129. Lees JS, Mark PB. Fruit for thought: lifestyle interventions to reduce the risk of future chronic kidney disease. *Nephrol Dial Transplant*. 2021;36(6):963-5
130. Kelly JT, Su G, Zhang L, Qin X, Marshall S, González-Ortiz A, et al. Modifiable Lifestyle Factors for Primary Prevention of CKD: A Systematic Review and Meta-Analysis. *J Am Soc Nephrol*. 2021;32(1):239-53
131. Bach KE, Kelly JT, Palmer SC, Khalesi S, Strippoli GFM, Campbell KL. Healthy Dietary Patterns and Incidence of CKD: A Meta-Analysis of Cohort Studies. *Clin J Am Soc Nephrol*. 2019;14(10):1441-9
132. Kogon AJ, Roem J, Schneider MF, Mitsnefes MM, Zemel BS, Warady BA, et al. Associations of body mass index (BMI) and BMI change with progression of chronic kidney disease in children. *Pediatr Nephrol*. 2023;38(4):1257-66
133. Chagnac A, Weinstein T, Herman M, Hirsh J, Gaftor U, Ori Y. The effects of weight loss on renal function in patients with severe obesity. *J Am Soc Nephrol*. 2003;14(6):1480-6
134. Friedman AN, Moe S, Fadel WF, Inman M, Mattar SG, Shihabi Z, et al. Predicting the glomerular filtration rate in bariatric surgery patients. *Am J Nephrol*. 2014;39(1):8-15
135. Lieske JC, Collazo-Clavell ML, Sarr MG, Rule AD, Bergstralh EJ, Kumar R. Gastric bypass surgery and measured and estimated GFR in women. *Am J Kidney Dis*. 2014;64(4):663-5
136. Navarro-Díaz M, Serra A, Romero R, Bonet J, Bayés B, Homs M, et al. Effect of drastic weight loss after bariatric surgery on renal parameters in extremely obese patients: long-term follow-up. *J Am Soc Nephrol*. 2006;17(12 Suppl 3):S213-7
137. Serra A, Granada ML, Romero R, Bayés B, Cantón A, Bonet J, et al. The effect of bariatric surgery on adipocytokines, renal parameters and other cardiovascular risk factors in severe and very severe obesity: 1-year follow-up. *Clin Nutr*. 2006;25(3):400-8
138. Ruggenenti P, Abbate M, Ruggiero B, Rota S, Trillini M, Aparicio C, et al. Renal and Systemic Effects of Calorie Restriction in Patients With Type 2 Diabetes With Abdominal Obesity: A Randomized Controlled Trial. *Diabetes*. 2017;66(1):75-86
139. Liu DJX, Stock E, Broeckx BJG, Daminet S, Meyer E, Delanghe JR, et al. Weight-gain induced changes in renal perfusion assessed by contrast-enhanced ultrasound precede increases in urinary protein excretion suggestive of glomerular and tubular injury and normalize after weight-loss in dogs. *PLoS One*. 2020;15(4):e0231662
140. Xiao N, Devarajan P, Inge TH, Jenkins TM, Bennett M, Mitsnefes MM. Subclinical kidney injury before and 1 year after bariatric surgery among adolescents with severe obesity. *Obesity (Silver Spring)*. 2015;23(6):1234-8
141. Inge TH, Courcoulas AP, Jenkins TM, Michalsky MP, Helmrath MA, Brandt ML, et al. Weight Loss and Health Status 3 Years after Bariatric Surgery in Adolescents. *N Engl J Med*. 2016;374(2):113-23
142. Nehus EJ, Khoury JC, Inge TH, Xiao N, Jenkins TM, Moxey-Mims MM, et al. Kidney outcomes three years after bariatric surgery in severely obese adolescents. *Kidney Int*. 2017;91(2):451-8
143. Bjornstad P, Nehus E, Jenkins T, Mitsnefes M, Moxey-Mims M, Dixon JB, et al. Five-year kidney outcomes of bariatric surgery differ in severely obese adolescents and adults with and without type 2 diabetes. *Kidney Int*. 2020;97(5):995-1005
144. Pottel H, Delanaye P, Schaeffner E, Dubourg L, Eriksen BO, Melsom T, et al. Estimating glomerular filtration rate for the full age spectrum from serum creatinine and cystatin C. *Nephrol Dial Transplant*. 2017;32(3):497-507

145. Moriconi D, Nannipieri M, Dadson P, Rosada J, Tentolouris N, Rebelos E. The Beneficial Effects of Bariatric-Surgery-Induced Weight Loss on Renal Function. *Metabolites*. 2022 Oct 12;12(10):967
146. Davidson LE, Yu W, Goodpaster BH, DeLany JP, Widen E, Lemos T, et al. Fat-Free Mass and Skeletal Muscle Mass Five Years After Bariatric Surgery. *Obesity (Silver Spring)*. 2018;26(7):1130-6
147. Chang AR, Chen Y, Still C, Wood GC, Kirchner HL, Lewis M, et al. Bariatric surgery is associated with improvement in kidney outcomes. *Kidney Int*. 2016;90(1):164-71
148. Shulman A, Peltonen M, Sjöström CD, Andersson-Assarsson JC, Taube M, Sjöholm K, et al. Incidence of end-stage renal disease following bariatric surgery in the Swedish Obese Subjects Study. *Int J Obes (Lond)*. 2018;42(5):964-73
149. Friedman AN, Wahed AS, Wang J, Courcoulas AP, Dakin G, Hinojosa MW, et al. Effect of Bariatric Surgery on CKD Risk. *J Am Soc Nephrol*. 2018;29(4):1289-300
150. Clerte M, Wagner S, Carette C, Brodin-Sartorius A, Vilaine É, Alvarez JC, et al. The measured glomerular filtration rate (mGFR) before and 6 months after bariatric surgery: A pilot study. *Nephrol Ther*. 2017;13(3):160-7
151. Solini A, Seghieri M, Santini E, Giannini L, Biancalana E, Taddei S, et al. Renal Resistive Index Predicts Post-Bariatric Surgery Renal Outcome in Nondiabetic Individuals with Severe Obesity. *Obesity (Silver Spring)*. 2019;27(1):68-74
152. Davis S, Nehus E, Inge T, Zhang W, Setchell K, Mitsnefes M. Effect of bariatric surgery on urinary sphingolipids in adolescents with severe obesity. *Surg Obes Relat Dis*. 2018;14(4):446-51
153. Bjornstad P, Nehus E, van Raalte D. Bariatric surgery and kidney disease outcomes in severely obese youth. *Semin Pediatr Surg*. 2020;29(1):150883
154. Mann JFE, Ørsted DD, Brown-Frandsen K, Marso SP, Poulter NR, Rasmussen S, et al. Liraglutide and Renal Outcomes in Type 2 Diabetes. *N Engl J Med*. 2017;377(9):839-48
155. Verma S, McGuire DK, Bain SC, Bhatt DL, Leiter LA, Mazer CD, et al. Effects of glucagon-like peptide-1 receptor agonists liraglutide and semaglutide on cardiovascular and renal outcomes across body mass index categories in type 2 diabetes: Results of the LEADER and SUSTAIN 6 trials. *Diabetes Obes Metab*. 2020 Dec;22(12):2487-2492
156. Gerstein HC, Colhoun HM, Dagenais GR, Diaz R, Lakshmanan M, Pais P, et al. Dulaglutide and renal outcomes in type 2 diabetes: an exploratory analysis of the REWIND randomised, placebo-controlled trial. *Lancet*. 2019;394(10193):131-8
157. Perkovic V, Tuttle KR, Rossing P, Mahaffey KW, Mann JFE, Bakris G, et al. Effects of Semaglutide on Chronic Kidney Disease in Patients with Type 2 Diabetes. *N Engl J Med*. 2024;391(2):109-21
158. Pi-Sunyer X, Astrup A, Fujioka K, Greenway F, Halpern A, Krempf M, et al. A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. *N Engl J Med*. 2015;373(1):11-22
159. Wilding JPH, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, et al. Once-Weekly Semaglutide in Adults with Overweight or Obesity. *N Engl J Med*. 2021;384(11):989-1002
160. Lincoff AM, Brown-Frandsen K, Colhoun HM, Deanfield J, Emerson SS, Esbjerg S, et al. Semaglutide and Cardiovascular Outcomes in Obesity without Diabetes. *N Engl J Med*. 2023;389(24):2221-32
161. Apperloo EM, Gorris JL, Soler MJ, Cigarrán Guldreis S, Cruzado JM, Puchades MJ, et al. Semaglutide in patients with overweight or obesity and chronic kidney disease without diabetes: a randomized double-blind placebo-controlled clinical trial. *Nat Med*. 2025 Jan;31(1):278-285
162. Badve SV, Bilal A, Lee MMY, Sattar N, Gerstein HC, Ruff CT, et al. Effects of GLP-1 receptor agonists on kidney and cardiovascular disease outcomes: a meta-analysis of randomised controlled trials. *Lancet Diabetes Endocrinol*. 2025 Jan;13(1):15-28

163. Kelly AS, Auerbach P, Barrientos-Perez M, Gies I, Hale PM, Marcus C, et al. A Randomized, Controlled Trial of Liraglutide for Adolescents with Obesity. *N Engl J Med*. 2020;382(22):2117-28
164. Weghuber D, Barrett T, Barrientos-Pérez M, Gies I, Hesse D, Jeppesen OK, et al. Once-Weekly Semaglutide in Adolescents with Obesity. *N Engl J Med*. 2022;387(24):2245-57
165. Katole NT, Salankar HV, Khade AM, Kale JS, Bankar NJ, Gosavi P, et al. The Antiobesity Effect and Safety of GLP-1 Receptor Agonist in Overweight/Obese Adolescents Without Diabetes Mellitus: A Systematic Review and Meta-Analysis. *Cureus*. 2024;16(8):e66280
166. Fox CK, Barrientos-Pérez M, Bomberg EM, Dcruz J, Gies I, Harder-Lauridsen NM, et al. Liraglutide for Children 6 to <12 Years of Age with Obesity - A Randomized Trial. *N Engl J Med*. 2025;392(6):555-65
167. Tamborlane WV, Laffel LM, Shehadeh N, Isganaitis E, Van Name M, Ratnayake J, et al. Efficacy and safety of dapagliflozin in children and young adults with type 2 diabetes: a prospective, multicentre, randomised, parallel group, phase 3 study. *Lancet Diabetes Endocrinol*. 2022;10(5):341-50
168. Shehadeh N, Barrett T, Galassetti P, Karlsson C, Monyak J, Iqbal N, et al. Dapagliflozin or Saxagliptin in Pediatric Type 2 Diabetes. *NEJM Evid*. 2023;2(12):EVIDoa2300210
169. Laffel LM, Danne T, Klingensmith GJ, Tamborlane WV, Willi S, Zeitler P, et al. Efficacy and safety of the SGLT2 inhibitor empagliflozin versus placebo and the DPP-4 inhibitor linagliptin versus placebo in young people with type 2 diabetes (DINAMO): a multicentre, randomised, double-blind, parallel group, phase 3 trial. *Lancet Diabetes Endocrinol*. 2023;11(3):169-81
170. Cai X, Yang W, Gao X, Chen Y, Zhou L, Zhang S, et al. The Association Between the Dosage of SGLT2 Inhibitor and Weight Reduction in Type 2 Diabetes Patients: A Meta-Analysis. *Obesity (Silver Spring)*. 2018;26(1):70-80
171. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med*. 2015;373(22):2117-28
172. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N Engl J Med*. 2019;380(24):2295-306
173. Zheng H, Liu M, Li S, Shi Q, Zhang S, Zhou Y, et al. Sodium-Glucose Co-Transporter-2 Inhibitors in Non-Diabetic Adults With Overweight or Obesity: A Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne)*. 2021;12:706914
174. Impact of diabetes on the effects of sodium glucose co-transporter-2 inhibitors on kidney outcomes: collaborative meta-analysis of large placebo-controlled trials. *Lancet*. 2022;400(10365):1788-801
175. Liu J, Cui J, Fang X, Chen J, Yan W, Shen Q, et al. Efficacy and Safety of Dapagliflozin in Children With Inherited Proteinuric Kidney Disease: A Pilot Study. *Kidney Int Rep*. 2022;7(3):638-41
176. Choi N, Kim JH, Park PG, Lee H, Min J, Park HW, et al. Efficacy and safety of dapagliflozin in children with kidney disease: real-world data. *Pediatr Nephrol*. 2024;39(12):3551-8
177. Gross O, Haffner D, Schaefer F, Weber LT. SGLT2 inhibitors: approved for adults and cats but not for children with CKD. *Nephrol Dial Transplant*. 2024;39(6):907-9
178. Kanbay M, Copur S, Bakir CN, Covic A, Ortiz A, Tuttle KR. Glomerular hyperfiltration as a therapeutic target for CKD. *Nephrol Dial Transplant*. 2024;39(8):1228-38
179. Adebayo OC, Nkoy AB, van den Heuvel LP, Labarque V, Levchenko E, Delanaye P, et al. Glomerular hyperfiltration: part 2-clinical significance in children. *Pediatr Nephrol*. 2023;38(8):2529-47

A

Addendum

SUMMARY

Obesity is a chronic disease characterized by excessive fat deposits that harms health, and its prevalence in children has risen majorly over the last decades. As a major risk factor for chronic kidney disease (CKD), obesity is an important modifiable factor in the prevention and deterioration of CKD. The major aims of this thesis were to 1) improve understanding of serum creatinine (SCr) and SCr-based estimated glomerular filtration rate (eGFR) equations in children with overweight and obesity without kidney disease, 2) explore the relation between eGFR and obesity-related comorbidities in childhood obesity, and 3) assess the effect of a multidisciplinary lifestyle intervention on SCr and eGFR in children with overweight and obesity.

Estimation of GFR in children with overweight and obesity

As a key marker of kidney function, GFR is commonly estimated using SCr. Studies on SCr in children with overweight and obesity show inconsistent results, possibly due to confounding factors (e.g. age, height, and sex) and heterogeneous SCr-measurement methods. In **Chapter 2 and 3**, SCr and SCr-based eGFR were analysed in 600 children with overweight and obesity without CKD. Enzymatically measured SCr was 'rescaled' using Q-age and Q-height (the median SCr value of a child with corresponding age (and sex in case of an age >14 years) or height, respectively), resulting in SCr/Q. Most children (96.5%) had SCr values within the SCr/Q-age reference range of [0.67-1.33] (corresponding to the 2.5th and 97.5th percentile, which equals eGFR FAS-age of about 81-160 ml/min/1.73m²).

In **Chapter 2**, commonly used SCr-based eGFR equations in children were compared. Since SCr values of children with overweight and obesity are mostly within the reference range and measured GFR is known to remain stable from age 2 to 40 years in healthy individuals, a flat and sex-independent GFR-age relationship is expected in children with overweight and obesity. Considering the distribution of eGFR, absence of age-dependency and order of magnitude of sex-dependency, eGFR FAS-age, FAS-height and LMR18 are suggested as the preferred eGFR equations in children with overweight and obesity aged 2 years and older.

In **Chapter 3**, SCr/Q is suggested to be a valuable add-on next to SCr-based eGFR equations. Advantages over eGFR equations include no modelling biases, a clear target value (SCr/Q = 1) and defined reference interval [0.67 - 1.33], independent from age and sex, and independence from BSA-adjustment.

Relation between eGFR and obesity-related comorbidities in childhood

In **Chapter 3**, correlations between eGFR, SCr/Q, and anthropometrical and metabolic variables were analysed in 600 children with overweight and obesity without CKD. SCr/Q

and some eGFR equations showed weak correlations with BMI z-score, fat mass, waist-to-hip ratio, HOMA-IR and HDL-cholesterol, triacylglyceride, serum uric acid and ALT concentrations (univariate correlation coefficients ranged from 0.1 to 0.3). Our findings support the role of disturbed body composition and other components of the metabolic syndrome in the pathogenesis of obesity-related kidney disease. Besides, it is clear that the choice of a SCr-based eGFR-equation influences observed correlations.

In **Chapter 4**, blood pressure (BP) was assessed in children with overweight and obesity, since hypertension is a common comorbidity in childhood obesity and a key risk factor for CKD. Less than half of the children had a normal BP measurement, and 24%, 25% and 6.0% of the children had an elevated BP measurement, stage 1 and stage 2 hypertensive BP measurement, respectively. Contrary to other studies, BMI z-score did not differ significantly between the BP categories. Weak positive correlations were found between eGFR and both systolic and diastolic BP percentile.

In **Chapter 5**, glucose dysregulation was examined in 106 children with overweight and obesity. Like hypertension, glucose dysregulation is common in children with overweight and obesity, and a key risk factor for CKD. Different stages of glucose dysregulation were already highly present: 56.6% of the children had a HOMA-IR > 2.5 (suggesting insulin resistance), and 23.6% and 3.8% of the children had prediabetes and impaired glucose tolerance, respectively. During 48-hour real-time continuous glucose monitoring (rtCGM), hyperglycemic sensor glucose excursions were present in 26.4% of the children with overweight and obesity. In children with overweight and obesity with suspected insulin resistance and hyperglycemic glucose excursions, time spent in hyperglycemic range in free living conditions is strongly positively correlated with SCr-based eGFR and the inverse of SCr/Q. These findings support the hypothesis of hyperglycemia-induced hyperfiltration in children with overweight and obesity.

Effect of lifestyle intervention on SCr, SCr/Q and eGFR in children with overweight and obesity

Lifestyle modification is the cornerstone in the treatment of childhood obesity and preventing CKD. While studies among adults suggest that obesity-related kidney disease and/or glomerular hyperfiltration may be reversible following weight loss, data in children are limited. In **Chapter 6 and 7**, the effect of the multidisciplinary lifestyle intervention program on SCr and eGFR in children with overweight and obesity are described.

After one year lifestyle intervention (**Chapter 6**), BMI z-score, waist-to-hip ratio, diastolic BP, HbA1c concentration and dyslipidemia improved significantly. SCr and SCr/Q increased, and eGFR (unit: ml/min/1.73m²) decreased significantly. De-indexing eGFR using actual BSA (unit: ml/min) negated this effect. In children with estimated glomerular

hyperfiltration, de-indexed eGFR decreased significantly. Changes in eGFR were not associated with changes in BMI z-score or other parameters of the metabolic syndrome.

During 5 years lifestyle intervention (**Chapter 7**), SCr/Q increased slightly each year (0.01-0.04), corresponding a small eGFR-FAS reduction (1.1-4.1 ml/min/1.73m²). BMI z-score decreased in both females and males and this reduction was significantly higher in males. Changes in BMI z-score were not correlated with SCr/Q changes. Moreover, there was no difference in the change of SCr/Q during follow-up between children who achieved a significant reduction in BMI z-score and those who did not. The observed SCr/Q increase (and eGFR decrease) may reflect increased muscle mass, altered tubular handling of SCr, and/or reversal of glomerular hyperfiltration.

Conclusions

In this thesis, the majority of children with overweight and obesity had a normal SCr-based eGFR and in these children aged 2 years and older, eGFR FAS-age, FAS-height and LMR18 can be allotted as the preferred eGFR equations. Associations were found between adiposity, eGFR, high blood pressure and impaired glucose regulation, supporting the role of disturbed body composition and other components of the metabolic syndrome in the pathogenesis of obesity-related kidney disease. During lifestyle intervention, a yearly SCr/Q increase of about 0.01-0.04 was demonstrated, which equals an eGFR-FAS reduction of about 1.1-4.1 ml/min/1.73m². Whether this minor change in eGFR is a favorable outcome and has clinical consequences on the long-term, requires further research.

In conclusion, current evidence suggests that excess weight in children puts the kidneys under pressure. (Pediatric) nephrologists should consider obesity as a modifiable risk factor for the development and progression of kidney disease.

SAMENVATTING

Obesitas is een chronische aandoening die wordt gekenmerkt door overmatige vetophoping die schadelijk is voor de gezondheid. De prevalentie van obesitas op de kinderleeftijd is de afgelopen decennia sterk toegenomen. Als belangrijke risicofactor voor chronische nierschade (chronic kidney disease, CKD) vormt obesitas een wezenlijke, beïnvloedbare determinant in zowel de preventie als de progressie van CKD.

De belangrijkste doelstellingen van dit proefschrift waren driedelig. Ten eerste werd het inzicht in serum kreatinine (SCr) en SCr-gebaseerde schattingen voor de glomerulaire filtratiesnelheid (estimated glomerular filtration rate, eGFR) bij kinderen met overgewicht en obesitas zonder bekende nierziekte verbeterd. Ten tweede werd de relatie tussen eGFR en obesitas-gerelateerde comorbiditeiten bij kinderen met overgewicht en obesitas onderzocht. Ten derde werd het effect van een multidisciplinaire leefstijlinterventie op SCr en eGFR bij kinderen met overgewicht en obesitas geëvalueerd.

Schatting van de GFR bij kinderen met overgewicht en obesitas

Als belangrijke maat voor de nierfunctie wordt de GFR doorgaans geschat op basis van SCr. Studies naar SCr bij kinderen met overgewicht en obesitas laten inconsistente resultaten zien, mogelijk als gevolg van versturende factoren (zoals leeftijd, lengte en geslacht) en heterogene methoden voor SCr-bepaling. In **hoofdstuk 2 en 3** werden SCr en SCr-gebaseerde eGFR geanalyseerd bij 600 kinderen met overgewicht en obesitas zonder bekende nierziekte. Enzymatisch gemeten SCr-waarden werden hierbij 'geschaald' met behulp van Q-age en Q-height (de mediane SCr-waarde van een kind met een overeenkomstige leeftijd (en bij een leeftijd >14 jaar ook geslacht) of lengte, respectievelijk), resulterend in de verhouding SCr/Q. Bij het merendeel van de kinderen (96,5%) lagen de SCr-waarden binnen het SCr/Q leeftijdsreferentie-interval van [0,67–1,33], overeenkomend met het 2,5^e en 97,5^e percentiel en een eGFR FAS-age van ongeveer 81–160 ml/min/1,73 m².

In **hoofdstuk 2** werden veelgebruikte, SCr-gebaseerde eGFR-formules voor kinderen met elkaar vergeleken. Deze studie toont aan dat SCr-waarden bij kinderen met overgewicht en obesitas doorgaans binnen het referentiegebied vallen. Daarnaast is bekend dat de gemeten GFR bij gezonde individuen stabiel blijft tussen het tweede en veertigste levensjaar. Daarom wordt bij kinderen met overgewicht en obesitas een vlakke en geslachts-onafhankelijke relatie tussen GFR en leeftijd verwacht. Op basis van de verdeling van eGFR, het ontbreken van leeftijdsafhankelijkheid en de orde van grootte van geslachtsverschillen worden eGFR FAS-age, FAS-height en LMR18 voorgesteld als de voorkeursformules voor het schatten van GFR bij kinderen met overgewicht en obesitas vanaf de leeftijd van twee jaar.

In **hoofdstuk 3** wordt SCr/Q voorgesteld als een waardevolle aanvullende parameter naast bestaande SCr-gebaseerde eGFR-formules. SCr/Q biedt meerdere voordelen ten opzichte van eGFR-formules. Ten eerste vermijdt dit bias die ontstaat als gevolg van het modelleren van SCr in een eGFR-formule. Ten tweede is er een duidelijke streefwaarde ($\text{SCr}/\text{Q} = 1$) en een goed gedefinieerd referentie-interval [0,67–1,33], dat onafhankelijk is van leeftijd en geslacht. Ten derde is SCr/Q onafhankelijk van correctie voor het lichaamsoppervlak.

Relatie tussen eGFR en obesitas-gerelateerde comorbiditeiten op de kinderleeftijd

In **hoofdstuk 3** werden correlaties onderzocht tussen eGFR, SCr/Q en antropometrische en metabole variabelen bij 600 kinderen met overgewicht en obesitas zonder bekende nierziekte. Voor SCr/Q en enkele eGFR-formules werden zwakke correlaties gevonden met BMI-z-score, vetmassa, taille-heupverhouding, HOMA-IR, HDL-cholesterol, triglyceriden, serum urinezuur en ALAT-concentraties (univariate correlatiecoëfficiënten variërend van 0,1 tot 0,3). Deze bevindingen ondersteunen de rol van een verstoorde lichaamssamenstelling en andere componenten van het metabool syndroom in de pathogenese van obesitas-gerelateerde nierschade. Daarnaast blijkt dat de keuze voor een specifieke SCr-gebaseerde eGFR-formule van grote invloed is op de sterkte van de waargenomen correlaties.

In **hoofdstuk 4** werd de bloeddruk bij kinderen met overgewicht en obesitas onderzocht, aangezien hypertensie een veelvoorkomende comorbiditeit is bij obesitas in de kinderleeftijd en een belangrijke risicofactor vormt voor CKD. Minder dan de helft van de kinderen had een normale bloeddrukmeting; bij respectievelijk 24%, 25% en 6% van de kinderen werden metingen verricht passend bij de definities verhoogde bloeddruk, hypertensie stadium 1 en hypertensie stadium 2. In tegenstelling tot eerdere studies werd er geen significant verschil in BMI-z-score gevonden tussen de verschillende bloeddrukcategorieën. Wel werden zwakke positieve correlaties aangetoond tussen eGFR en zowel het systolische als diastolische bloeddrukpercentiel.

In **hoofdstuk 5** werd een verstoorde glucosehuishouding onderzocht bij 106 kinderen met overgewicht en obesitas. Net als hypertensie komt een verstoorde glucosehuishouding frequent voor in deze populatie en vormt dit een belangrijke risicofactor voor CKD. Verschillende stadia van verstoorde glucosehuishouding bleken reeds in hoge mate aanwezig: 56,6% van de kinderen had een HOMA-IR > 2,5, passend bij insulineresistentie, terwijl respectievelijk 23,6% en 3,8% van de kinderen prediabetes en gestoorde glucosetolerantie vertoonden. Tijdens 48-uurs real-time continue glucosemonitoring (rtCGM) werden hyperglycemische glucose-excursies geregistreerd bij 26,4% van de kinderen. Bij kinderen met vermoedelijke insulineresistentie en hyperglycemische episodes bleek dat de tijd die zij doorbrachten in het hyperglycemische bereik sterk

positief gecorreleerd was met SCr-gebaseerde eGFR en met de inverse waarde van SCr/Q. Deze bevindingen ondersteunen de hypothese van hyperglycemie-geïnduceerde hyperfiltratie bij kinderen met overgewicht en obesitas.

Effect van leefstijlinterventie op SCr, SCr/Q en eGFR bij kinderen met overgewicht en obesitas

Leefstijlmodificatie vormt de hoeksteen van de behandeling van obesitas in de kinderleeftijd en van de preventie van CKD. Hoewel studies bij volwassenen suggereren dat obesitas-gerelateerde nierschade en/of glomerulaire hyperfiltratie reversibel kunnen zijn na gewichtsreductie, zijn gegevens bij kinderen beperkt. In **hoofdstuk 6 en 7** wordt het effect beschreven van een multidisciplinair leefstijlinterventieprogramma op SCr, SCr/Q en eGFR bij kinderen met overgewicht en obesitas.

Na één jaar leefstijlinterventie (**hoofdstuk 6**) werden significante verbeteringen gezien in BMI-z-score, taille-heupverhouding, diastolische bloeddruk, HbA1c-concentratie en dyslipidemie. Zowel SCr als SCr/Q namen toe, terwijl eGFR (ml/min/1,73 m²) significant afnam. Na deïndexering van eGFR voor het werkelijke lichaamsoppervlak (ml/min) verdween dit effect. Bij kinderen met geschatte glomerulaire hyperfiltratie nam de gedeïndexeerde eGFR echter wel significant af. Veranderingen in eGFR correleerden niet met veranderingen in BMI-z-score of andere parameters van het metabool syndroom.

Gedurende vijf jaar leefstijlinterventie (**hoofdstuk 7**) nam SCr/Q jaarlijks licht toe (0,01–0,04), overeenkomend met een geringe afname van eGFR FAS van 1,1–4,1 ml/min/1,73 m². De BMI-z-score daalde zowel bij meisjes als jongens, waarbij deze afname significant groter was bij jongens. Veranderingen in BMI-z-score correleerden niet met veranderingen in SCr/Q. Bovendien werd geen verschil gevonden in de verandering van SCr/Q gedurende de follow-up tussen kinderen die een significante reductie in BMI-z-score bereikten en kinderen bij wie dit niet het geval was. De waargenomen toename van SCr/Q (en afname van eGFR) kan mogelijk worden verklaard door een toename van spiermassa, veranderingen in de tubulaire verwerking van SCr en/of reversibiliteit van glomerulaire hyperfiltratie.

Conclusies

In dit proefschrift had het merendeel van de kinderen met overgewicht en obesitas een normale SCr-gebaseerde eGFR. Bij kinderen van twee jaar en ouder kunnen eGFR FAS-age, FAS-height en LMR18 worden beschouwd als de voorkeursformules voor het schatten van de GFR. Daarnaast werden associaties gevonden tussen adipositas, eGFR, verhoogde bloeddruk en verstoorde glucosehuishouding, hetgeen de rol ondersteunt van een verstoorde lichaamssamenstelling en andere componenten van het metabool syndroom in de pathogenese van obesitas-gerelateerde nierschade. Tijdens leefstijlinterventie werd een jaarlijkse toename in SCr/Q van circa 0,01–0,04 waargenomen, overeenkomend

met een afname van eGFR FAS van ongeveer 1,1–4,1 ml/min/1,73 m². Of deze beperkte verandering in eGFR gunstig is en welke klinische consequenties dit op de lange termijn heeft, vereist nader onderzoek.

Concluderend suggereert het huidige bewijs dat overgewicht bij kinderen de nieren onder druk zet. (Kinder)nefrologen zouden obesitas moeten beschouwen als een beïnvloedbare risicofactor voor de ontwikkeling en progressie van nierziekten.

VALORISATION

This thesis describes and thereby draws attention to the association between childhood obesity and kidney disease, one of the ‘hidden consequences’ of the childhood obesity pandemic. In this chapter we describe the knowledge valorisation of the research presented in this thesis, focusing on the practical, social and economic aspects. In order to improve readability, in this chapter the terms ‘estimated glomerular filtration rate (eGFR)’ and ‘kidney function’ are used interchangeably.

Obesity is a well-established modifiable risk factor for the development and deterioration of kidney disease (1, 2). Not only obesity itself, but also its common comorbidities like hypertension and type 2 diabetes mellitus are well-known causes of chronic kidney disease (CKD) (3). Therefore, the global obesity pandemic is an extreme worrying situation for kidney health in the upcoming decades (1, 4). Some authors take this a step further and describe the obesity pandemic as the most important public health crisis the nephrology community is facing today (2). Indeed, CKD already has a global prevalence of about 850 million people, translating to a global prevalence of >10% (5). Moreover, this prevalence is probably an underestimate since there is a large global disparity in screening for kidney disease and a large proportion of cases go undetected and therefore uncounted (6). Kidney disease is the third-fastest-growing cause of death globally and by 2040, it is expected to become the fifth-highest cause of years of life lost (4, 7).

Kidney disease causes premature mortality and the burden of symptoms of CKD on individuals is profound (7). Children and adults with CKD experience a similar or greater symptom burden than those with malignancies and type 1 diabetes mellitus (8, 9). Besides, the direct and indirect health care costs of CKD are relevant at the global, national, health system and individual levels. Treatment of kidney disease presents many challenges and account for a disproportionately large amount of national economic expenditure (7). In Europe for example, aggregated annual health care costs of CKD are estimated to be higher than those of malignancies or diabetes mellitus (10).

Childhood-onset obesity is associated with CKD and kidney-related mortality in adulthood (2). Therefore, the treatment of the childhood obesity pandemic can be considered as an effective method in preventing future CKD. Since curative treatments for CKD to date do not exist, all profit can be achieved from prevention. It has been predicted that up to 33% of kidney disease in the United States could be prevented if overweight and obesity were eliminated (11). Considering this, it is surprising that the negative consequences of obesity on kidney health has long not been given priority and it is only since the last decades that obesity-related kidney disease gets appropriate attention (1). Recently, a joint statement from, among others, the International Society of Nephrology, European Renal Association and American Society of Nephrology advocates

for the inclusion of kidney disease in the current World Health Organization statement on major non-communicable disease drivers of premature mortality (7).

Still, research on kidney function in children with overweight and obesity is scarce. The results from this thesis contribute to our understanding how best to assess kidney function in children with overweight and obesity and how and to what extent obesity and its comorbidities are associated with kidney function in children. Moreover, the longitudinal studies in this thesis are, to the best of our knowledge, the first studies in children with overweight and obesity in which the effects of a multidisciplinary lifestyle intervention, the first step in the treatment of childhood obesity (12), on kidney function are evaluated.

It is beyond dispute that overweight and obesity in children has devastating somatic, psychosocial and social consequences that extend way beyond kidney health, and some of the results in this thesis contribute to our understanding of childhood obesity in general.

Obesity is an independent risk factor for the development and progression of CKD and besides, obesity is known to aggravate primary kidney diseases. In other words: every child and adult with overweight or obesity has an increased risk of developing CKD. Since CKD is a condition that worsens subclinically, without screening, individuals often present in high CKD stages with multiple comorbidities (like hypertension, anemia, and mineral bone disease). So, every healthcare provider, not in the least those working in primary health care, needs to consider individuals with overweight and obesity at risk for kidney disease. Moreover, in every patient with a congenital or acquired kidney disease, this is for example well-established in patients with a solitary functioning kidney (13) and autosomal dominant polycystic kidney disease (14), obesity has to be considered as a modifiable risk factor. Since a subgroup of nephrology patients are at risk of developing obesity resulting from their treatment (e.g. steroids) or energy misbalance resulting from disease burden (e.g. fatigue), the obesity-kidney relation can act in both ways. Most important though: society, and thus individuals with overweight and obesity themselves, has to be aware of the devastating consequences of overweight and obesity on kidney health.

This thesis also contributes to improvements in daily practice. Included in this thesis is the evaluation of multiple eGFR equations in children with overweight and obesity. These GFR-estimating equations make it possible to convert an endogenous marker (most common: serum creatinine) into an eGFR value, that in turn is used for evaluation of possible acute kidney injury, CKD staging, drug dosing etc. Since presenting physicians with eGFR, instead of serum creatinine values, can dramatically improve the ability of the medical team to assess kidney function correctly in children (15), notification of eGFR in

laboratory results is of major importance. It was not until December 9th 2024 that such eGFR notification exists for children in the laboratory result in Maastricht University Medical Centre + MosaKids Children's hospital. Based on the knowledge obtained from this thesis, we implemented eGFR FAS-age (16), a solid choice in both healthy children and children with CKD, independent of weight category (as we have shown in Chapter 2 of this thesis). Even so, as was decided in a meeting of pediatric nephrologists throughout the Netherlands, eGFR FAS-age will be the eGFR equation of first choice for children in all (university) hospitals.

Early detection is a key strategy to prevent kidney disease, its progression and related complications. Considering obesity as an important modifiable risk factors for the development and progression of kidney disease, (pediatric) nephrologists should routinely assess obesity as part of their clinical management. Realizing the difficulties in the treatment of children with obesity, due to its multifaceted etiology, we recommend that pediatric nephrologist familiarize themselves with the regional networks available for supporting children with overweight and obesity and their families in achieving a healthier weight and lifestyle. In this way, lifestyle intervention can be guided by professionals in the field, ensuring high-quality and appropriate support. It is important to recognize that the guidance of children with overweight and obesity is a specialized profession in its own right. In 2025, the Dutch Healthcare Authority (NZa) set the rates for the total costs for three years of a combined lifestyle intervention for children with overweight and obesity at approximately €2000 (17). Assuming that the implementation of a combined lifestyle intervention in children with overweight and obesity will contribute to a reduction in obesity-related comorbidities, including CKD, in the future, this investment is expected to lead to a decrease in the overall annual healthcare costs associated with overweight and obesity.

In conclusion, this research into the effects of overweight and obesity during childhood on the kidneys is highly significant due to the far-reaching impact these conditions have on health at various levels. The relevance of this research is thus of great importance both clinically and socio-economical. We cannot stress enough the physical and social impact of CKD on individuals and families and the major public health challenges obesity-related kidney disease entails. In order to possibly prevent kidney disease, the pediatric nephrology community should take an active role in supporting policies that address the current obesogenic environment and encourage healthy lifestyle practices among children.

REFERENCES

1. Kovesdy CP, Furth SL, Zoccali C. Obesity and kidney disease: hidden consequences of the epidemic. *Pediatr Nephrol*. 2017;32(4):537-45
2. Nehus E, Mitsnefes M. Kidney consequences of obesity. *Pediatr Nephrol*. 2025 Jun;40(6):1879-1893
3. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. *Lancet*. 2017;389(10075):1238-52
4. Time to sound the alarm about the hidden epidemic of kidney disease. *Nature*. 2024;628(8006):7-8
5. Jager KJ, Kovesdy C, Langham R, Rosenberg M, Jha V, Zoccali C. A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases. *Nephrol Dial Transplant*. 2019;34(11):1803-5
6. Bello AK, Okpechi IG, Levin A, Ye F, Damster S, Arruebo S, et al. An update on the global disparities in kidney disease burden and care across world countries and regions. *Lancet Glob Health*. 2024;12(3):e382-e95
7. Francis A, Harhay MN, Ong ACM, Tummalapalli SL, Ortiz A, Fogo AB, et al. Chronic kidney disease and the global public health agenda: an international consensus. *Nat Rev Nephrol*. 2024;20(7):473-85
8. Kalantar-Zadeh K, Lockwood MB, Rhee CM, Tantisattamo E, Andreoli S, Balducci A, et al. Patient-centred approaches for the management of unpleasant symptoms in kidney disease. *Nat Rev Nephrol*. 2022;18(3):185-98
9. Francis A, Didsbury MS, van Zwieten A, Chen K, James LJ, Kim S, et al. Quality of life of children and adolescents with chronic kidney disease: a cross-sectional study. *Arch Dis Child*. 2019;104(2):134-40
10. Vanholder R, Annemans L, Bello AK, Bikbov B, Gallego D, Gansevoort RT, et al. Fighting the unbearable lightness of neglecting kidney health: the decade of the kidney. *Clin Kidney J*. 2021;14(7):1719-30
11. Wang Y, Chen X, Song Y, Caballero B, Cheskin LJ. Association between obesity and kidney disease: a systematic review and meta-analysis. *Kidney Int*. 2008;73(1):19-33
12. Hampl SE, Hassink SG, Skinner AC, Armstrong SC, Barlow SE, Bolling CF, et al. Clinical Practice Guideline for the Evaluation and Treatment of Children and Adolescents With Obesity. *Pediatrics*. 2023;151(2)
13. Groen In 't Woud S, Roeleveld N, Westland R, Renkema KY, Steffens MG, Gracchi V, et al. Uncovering risk factors for kidney injury in children with a solitary functioning kidney. *Kidney Int*. 2023 Jan;103(1):156-165
14. Nowak KL, You Z, Gitomer B, Brosnahan G, Torres VE, Chapman AB, et al. Overweight and Obesity Are Predictors of Progression in Early Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol*. 2018;29(2):571-8
15. Schnapp A, Egger Y, Bignall ONR, 2nd, Issler N, Volovelsky O. Estimated pediatric glomerular filtration rate presentation improves the detection rate of kidney impairment in children. *Pediatr Nephrol*. 2023;38(9):3091-7
16. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol*. 2012;27(6):973-9
17. Dutch Healthcare Authority (NZa). Structurele bekostiging voor de aanpak van kinderen met overgewicht en obesitas. TB/REG-25604-01. 2025. Available from: https://puc.overheid.nl/nza/doc/PUC_759588_22/.

DANKWOORD

De weg is het doel, maar wat kan het behalen van de eindstreep toch fijn zijn. Wat begon als een wetenschapsstage binnen COACH Maastricht als student geneeskunde, is uitgegroeid tot een proefschrift als kinderarts-nefroloog. Deze reis had ik nooit alleen kunnen maken en hiervoor ben ik veel mensen dankbaar. Ik kan lang niet iedereen bij naam noemen, zal belangrijke personen niet vermelden en zelfs in de korte beschrijvingen geef ik velen niet de eer die zij verdienen. Hierbij mijn poging:

Allereerst de patiënten en hun ouders/verzorgers. Jullie laten mij elke dag weer zien waar ik dit werk voor doe.

Prof. dr. Anita Vreugdenhil, beste Anita, we kennen elkaar nu vrijwel tien jaar en wat is er in de tussentijd een hoop gebeurd. Van kinderarts maag-, darm- en leverziekten tot grondlegger van COACH Maastricht, waarbij de COACH-methodiek nu ook in de rest van Nederland wordt ingezet om kinderen met overgewicht en obesitas een meer dan goede zet in de rug te geven. Je harde werk is onder andere beloond met een hoogleraarschap en ik ben benieuwd waar jouw reis nog allemaal naartoe gaat. Bedankt dat je mij de kans hebt gegeven om mijn eigen weg te bewandelen en bedankt dat ik de COACH-data mocht gebruiken voor mijn onderzoek.

Em. Prof. dr. Hans Pottel, beste Hans, na het lezen van het door u geschreven artikel 'Measuring and estimating glomerular filtration rate in children' (Pediatric Nephrology, 2017) besloot ik om advies aan u te vragen. Vrij snel daarna kwam u samen met Pierre Delanaye op bezoek in Maastricht, en ik had nooit durven dromen dat een dergelijk mailtje zou uitgroeien tot zo'n fantastische samenwerking. Ik had mij geen betere tweede promotor kunnen wensen. Ik ben u dankbaar voor de fijne samenwerking, voor de tijd die u heeft genomen mij te begeleiden en voor het geduld dat u heeft gehad met onze artikelen. En niet in het minst voor de geweldige verhalen in het prachtige Kortrijk en uw voorbeeldfunctie, ook in het begeleiden van studenten. Hoe bijzonder is het dat mijn carrière als kinderarts-nefroloog juist gestart is en uw glansrijke carrière bijna ten einde is. Hans, meer dan dank, zonder u was dit niet gelukt.

De leden van de beoordelingscommissie, prof. dr. Estela Rubio Gozalbo, dr. Arend Bökenkamp, prof. dr. Djalila Mekahli, prof. dr. Edgar van Mil, dank voor uw tijd, energie en kritische beoordeling van dit proefschrift.

Prof. dr. Gertjan Driessen, beste Gertjan, dank voor je vertrouwen in mij als kinderarts-nefroloog. Dank voor je luisterend oor, oprechtheid en steun. Het MUMC+ MosaKids Kinderziekenhuis heeft wat mij betreft in jou een uitstekend frontman. Ik hoop dat

we de komende jaren de zorg voor kinderen met en zonder nierziekte kunnen blijven optimaliseren.

Drs. Flore Engels, beste Flore, je hebt mijn reis van geneeskundestudent tot collega kinderarts-nefroloog mede gevormd en meegemaakt. Je was een van mijn coaches in het derde jaar van de geneeskundeopleiding, de eerste kinderarts-nefroloog met wie ik destijds een poli heb meegelopen, mijn vertrouwde gezicht tijdens de buitenlandse congressen en een van mijn opleiders tot kinderarts-nefroloog. Veel lof voor de manier waarop je nieuwe ambities niet uit de weg gaat. Ik bewonder de manier waarop je je werk als kinderarts-nefroloog combineert met jullie EBURON Estate: een waar paradijs.

Team kindernefrologie Radboudumc Amalia kinderziekenhuis:

Dr. Marlies Cornelissen, beste Marlies, opleider maar voor mij veel meer dan dat. Dank voor de vele lessen en voor het vertrouwen dat ik heb gekregen, zowel voorafgaand aan als tijdens mijn fellowship. Ik waardeer de manier waarop je mij hebt laten zien hoe je zorg van de hoogste kwaliteit levert, zonder daarbij ooit de aandacht voor de kinderen en hun ouders/verzorgers te verliezen. Daarnaast heb ik grote bewondering voor de manier waarop je de groep leidt. Je weet overzicht te bewaren en tegelijkertijd oog te hebben voor iedereen. Dat maakt je niet alleen een sterke leider en opleider, maar ook een heel prettig mens om mee samen te werken.

Femke Glaap, wat boft iedereen toch met jou. Je bent een vriendelijke duizendpoot: van urotherapie tot nierstenen, nierfalen en transplantatiezorg. Je bent het vaste gezicht voor kinderen en ouders/verzorgers en niets is je ooit te veel. Bedankt voor de fijne samenwerking.

Jacqueline Knoll, het is werkelijk fantastisch om te zien hoe jij je al die jaren al inzet voor kinderen met een nierziekte. Bedankt dat je mij als AIOS en nadien als fellow aan de hand hebt genomen, en bedankt voor je wijze lessen tijdens onder andere de dinsdagochtend (CAKUT) poli en transplantatiepoli.

Prof. dr. Nicole van de Kar, beste Nicole, mijn plaatsvervangend opleider tot kinderarts-nefroloog. Grote bewondering voor de manier waarop jij je strepen hebt verdiend binnen de complement wereld. Wat ik vooral in jou waardeer, is je oprechte hart voor de kinderen, wat hun ziektebeeld ook is. Ik heb veel van je geleerd tijdens mijn fellowship, leer ook nu nog steeds van je, en het is altijd erg prettig om met je samen te werken.

Drs. Linda Koster, beste Linda, wat heb ik veel van je mogen leren, niet in het minst op de dialyseafdeling. We hebben veel beleefd samen en wat hebben we ook veel lol gehad. Ik waardeer je precisie, kennis en kunde, je eerlijkheid en je hart voor de kinderen. Ook van

jou heb ik geleerd hoe je één team vormt met kinderen en hun ouders/verzorgers, en de collega's om je heen. Heel fijn dat we nog samenwerken.

Dr. Ilse Rood, beste Ilse, als internist-nefroloog was je plots onderdeel van het kindernefrologie team. Ik heb grote bewondering voor de manier waarop je vanaf dag één optimale zorg levert aan kinderen met een nierziekte en hun ouders/verzorgers.

Prof. dr. Michiel Schreuder, beste Michiel, ik durf gerust te beweren dat dit proefschrift er heel anders had uitgezien zonder jouw hulp. Je bent daarnaast onmisbaar geweest in mijn vorming tot kinderarts-nefroloog. Ik heb ontzettend veel van je mogen leren als dokter en wetenschapper. Wat ik vooral in jou waardeer is je benaderbaarheid, eerlijkheid en de manier waarop je (jongere) collega's begeleid. Dank voor het vertrouwen dat je altijd hebt gegeven. Van ons eerste gesprek in hotel Duinzicht tijdens Nier op Schier tot aan de inhoudelijke, en niet te vergeten mentale, ondersteuning in de laatste fase van dit proefschrift: je was er altijd en dacht op een fijne manier kritisch mee. Michiel, ontzettend bedankt.

Dr. Nynke Teeninga, beste Nynke, als net klare kinderarts-nefroloog nam het leven voor jou een nare wending en zie nu eens. Grote lof voor de manier waarop jij je momenteel inzet voor de kinderen met peritoneaal dialyse en voor het onderwijs.

Dr. Kioa Wijnsma, beste Kioa, wij kennen elkaar al sinds het ESPN congres in Glasgow in 2017 en zie ons nu: onze carrière-droom is werkelijkheid. Succes met je fellowship, voor mij ben je al kinderarts-nefroloog.

Drs. Karin van Dael, beste Karin, wat vind ik het jammer dat onze wegen elkaar net niet hebben gekruist in Maastricht, want wat had ik graag meer van je willen leren. Ik wil je bedanken voor je enthousiasme tijdens de ESPN en IPNA congressen, en niet in het minst voor je support tijdens mijn eerste internationale presentatie op de ESPN in Glasgow 2017. Fijn dat we elkaar nog vaker zien.

Kinderuroloog drs. Piet Callewaert, beste Piet, ik hoop dat we onze samenwerking verder kunnen optimaliseren en uitbreiden, in het belang van de kinderen, hun ouders/verzorgers, onze partners in de regio en voor onszelf.

Team kinderurologie in het Radboudumc Amalia kinderziekenhuis, dr. Emilie Goessens, dr. Barbara Kortmann, dr. Allon van Uitert en dr. Liesbeth de Wall. Dank voor de fijne samenwerking, zowel tijdens mijn fellowship als ook nu nog.

Collega's in het MUMC+ MosaKids Kinderziekenhuis: secretariaat kindergeneeskunde, poli-assistentes van het Vrouw, Moeder en Kind Centrum, stafleden, fellows en arts-

assistenten kindergeneeskunde, NICU en PICU, kinderverpleegkundigen, medisch pedagogisch team, PROSA-team, kinderpsychologen en alle anderen. Een bijzonder woord van dank aan Natascha voor de secretariële ondersteuning. Dank aan mijn oud fellow- en deels kamergenoten Anne, Else, Eva, Kirsten, Lizanne, Manouk, Rick en Sophie voor de fijne momenten. Tevens dank aan de leden van de werkgroep continue nierfunctievervangende therapie: Arno, Katleen, Katrien, Mieke en Mieke. Dat we samen deze therapie kunnen blijven optimaliseren en zo fijn met elkaar mogen blijven samenwerken.

Collega kinderartsen met aandachtsgebied nefrologie/urologie in de omringende ziekenhuizen: Caro, Eva, Guido, Hylke, Irene, Rick, Rob en alle anderen. Fijn om de zorg voor kinderen met een nierziekte samen te mogen dragen en, waar mogelijk, de zorg zo dicht mogelijk bij huis te kunnen bieden.

Collega's van de kinderradiologie, dank voor jullie hulp en flexibiliteit, ook bij het uitvoeren van de nierbipten. Dank ook aan de collega's van de prenatale diagnostiek en het neurale buisdefecten team. Collega's van de klinische genetica, fijn dat we nu nog intensiever samenwerken tijdens de nefrogenetica poli. Collega's van de interne nefrologie en vasculaire geneeskunde in het MUMC+, bedankt dat ik mij te allen tijde welkom voel om jullie hulp te vragen.

Collega's van de dialyse afdeling in het Radboud Amalia kinderziekenhuis, in het bijzonder: Alice, Barend, Carla, Christel, Dieuwke, Esmee, Femke, Marieke, Marlies, Milou, Oksana, Sylvia, Sylvie en Tijme. Dank voor de geweldige tijd tijdens mijn fellowship en voor de fijne samenwerking, ook nu nog. We hebben samen de uitersten binnen de zorg voor kinderen met een nierziekten beleefd, en dat hebben we altijd als team gedaan. Een team van zorgverleners én kinderen en hun ouders/verzorgers. Altijd serieus, maar met een luchtig randje; mooier wordt de zorg niet.

Collega's van de dialyse afdeling van het MUMC+, bedankt voor de fijne samenwerking. Ik hoop dat we deze de komende jaren kunnen gaan uitbreiden.

Dr. Pieter van Paassen en dr. Sjoerd Timmermans, dank voor jullie hulp bij kinderen met een immunologische nierziekte, jullie beoordelingen van de nierbipten en voor de prettige samenwerking. Tevens veel dank voor het inkijkje in jullie poliklinische zorg tijdens een deel van mijn fellowship. Sjoerd, dit hadden we toch niet kunnen dromen als jonge geneeskundestudenten: van 'immuno-boys' naar waar we nu staan. Dat we de komende jaren onze samenwerking mogen uitbreiden.

Dr. Pierre Delanaye, cher Pierre, nous vivons à environ 30 km l'un de l'autre, mais nous ne partageons pas la même langue maternelle. Merci pour votre aide lors de l'élaboration de

cette thèse ainsi que pour votre visite au MUMC+ à l'époque. Ce fut un réel plaisir d'avoir rédigé un article ensemble. J'espère que nous pourrons poursuivre cette collaboration.

Dr. Jamiu Busari, beste Jamiu, bedankt voor het vertrouwen in mij als geneeskundestudent. Ook wil ik je bedanken voor de medische, maar vooral ook de niet-medisch inhoudelijke lessen tijdens mijn semi-arts stage kindergeneeskunde in het Zuyderland Medisch Centrum in Heerlen (destijds nog Atrium MC geheten): het Eisenhower kwadrant speelt nog regelmatig door mijn hoofd. Je hebt geluisterd naar mijn ambities om kinderarts-nefroloog te worden, en waar anderen die destijds niet direct serieus leken te nemen, heb je er net als ik in geloofd en zie nu: het is zover. Ik hoop dat je strijd om gelijkheid steeds minder nodig zal zijn.

Oud-collega's binnen COACH Maastricht en mede ex-PhD studenten, in het bijzonder: Cara, Elke, Jesse, Johanna, Kylie, Marijn, Ronny en Yvon. Bedankt voor de vele fijne momenten en voor jullie steun.

Mijn vrienden:

Thijs, middelbare schoolvriend, de anekdotes zijn ontelbaar. Dit hadden we toch allemaal niet kunnen bedenken toen we zestien waren. Dat we elkaar, ondanks de afstand Friesland-Limburg, nog steeds zien en spreken, is fijn en bijzonder.

Giel, van studiegenoten werden we vrienden. We leerden elkaar beter kennen in India en zijn nu zelfs collega's. Dat we elkaar maar vaak mogen blijven zien, het is een genot om Robin, Rein en jou samen te zien.

Sven, ook wij waren studiegenoten en hebben India samen beleefd. Dat we samen, ook met Anneke, Femke en Sanne, nog maar veel avonturen mogen beleven.

Tan Lai, ik ken weinig mensen met zoveel humor, wonderlijke verhalen en hobby's. Momenten met jou zijn altijd bijzonder fijn.

Jacqueline, ook al zien we elkaar niet zo vaak, de basis voor onze vriendschap, gelegd tijdens onze studie en in India, is meer dan goed. Altijd fijn om samen met jou, Casper en Lars te zijn.

Ron en Jacqueline, van burens tot vrienden. Ontelbare spelletjesavonden, borrels, en vooral heel veel plezier. Dat we elkaar nog maar vaak mogen blijven zien.

Servaas, oud-bandlid, en vanaf die tijd (ongeveer twintig jaar geleden) vriend. We zien elkaar minder vaak dan we zouden willen. Dat we snel weer samen gaan kamperen (dit keer nemen we de tentstokken mee).

Dank aan Sytze, Mitch, Sabine en Nanette, voor alle gezelligheid. Jullie hebben mijn studententijd veel mooier gemaakt.

Etienne, hoe bijzonder is het: van middelbare school vrienden naar nu collega kinderartsen. Dat we nog maar lang mogen blijven samenwerken.

Leden van de Nestels, ons wandelgenootschap. Dat we ooit weer samenkomen en onze avonturen op onder andere het South West Coast Path en de Affric Kintail Way kunnen herbeleven.

Mijn familie:

Lieve Huub en Marianne, bedankt voor jullie liefde en steun. Toen ik als zeventienjarige voor het eerst bij jullie over de vloer kwam, hadden we dit alles toch nooit kunnen bedenken. Wat hebben we veel meegemaakt samen. Als opa Huub en Nonna genieten jullie intens van Elea, het is werkelijk fantastisch om dat te zien. Fijn dat jullie mijn schoonouders zijn.

Lieve Janeau en Lotte, ik geniet ervan te zien hoe jullie in het leven staan: drukke banen, hobby's genoeg en vooral veel gezelligheid. Bedankt voor jullie liefde.

Lieve Salina en Tristan, ook al wonen jullie (nog?) wat verder weg, we zien elkaar geregeld en dat is erg fijn. Dat we nog maar vaak mogen optreden als Sokker. Salina, bedankt voor je creaties, die koesteren we.

Lieve pap en mam, Jaap van Dam en Lydia van Dam - Frints (ja, jullie namen moeten in dit boekje), bedankt voor jullie onvoorwaardelijke liefde en steun. Ik weet dat ik altijd bij jullie terecht kan, wat er ook is. Het is fijn dat we dicht bij elkaar wonen en elkaar zo vaak zien, en ik geniet ervan te zien hoe fijn jullie en Elea het samen hebben. Als kinderarts besef ik me nog meer wat een geluk ik heb gehad met jullie als ouders. Bedankt dat jullie mijn ouders zijn.

Lieve Paul en Teun, mijn twee broers. Van jongs af aan hebben we een speciale band en dat we nu nog regelmatig met z'n drieën bij elkaar zijn, is toch geweldig. Ik ben trots op jullie en vereerd dat jullie mijn paranimfen zijn.

Lieve Lian, fijn dat je mijn schoonzus bent. Je staat altijd voor iedereen klaar, vol liefde en begrip. Wat hebben Paul, Saar, Fien en Tuur ontzettend veel geluk met jou. Mooi om te zien hoe jij je hart volgt.

Lieve Sandra, fijn dat je mijn schoonzus bent. Ik waardeer je creativiteit en rust, en hoe je het leven van Teun en jullie hond Ollie zo fijn maakt. Ik bewonder hoe jij met jouw lens verhalen vertelt.

Lieve Saar, Fien en Tuur, bij jullie op bezoek komen is altijd een feestje. Ik geniet ervan om te zien hoe jullie opgroeien en steeds meer jullie eigen, bijzondere zelf worden.

Lieve Marisya, alles wat ik hier schrijf, doet je tekort. We zijn nu twintig jaar bij elkaar, waarvan we al bijna zes jaar getrouwd zijn. Onze reis maken we samen en wat hebben we het fijn met elkaar, ook als het leven ons tegenzit. Mijn promotietraject was vol uitdagingen, en ik weet zeker dat het zonder jou nooit was gelukt. Bedankt voor je onvoorwaardelijke liefde, je eindeloze geduld en steun, en voor onze dochters.

Lieve Elea, drie jaar en al zo wijs. Je bent zo'n bijzonder en lief meisje. Ik ben elke dag dankbaar dat je in ons leven bent gekomen. Word maar niet te snel groot, en als dat toch gebeurt: volg je hart. Weet dat mama en ik je in alles zullen steunen.

Lieve Nine (†), we hebben je kort gezien en moesten je laten gaan. Mama en ik dragen je altijd met ons mee.

ABOUT THE AUTHOR

Mark Jacob Carla Marion van Dam was born on November 29, 1988 in Gouda, the Netherlands. He grew up in a close-knit family with his parents and brothers. After attending primary school Op 't Hwagveld in Meerssen, he continued his education at Stella Maris College in Meerssen, where he graduated in 2007. Following one year of Molecular Life Sciences at Maastricht University with cum laude results, he completed additional secondary education at ROC Leeuwenborgh in Maastricht, graduating cum laude. This strategic choice allowed him to bypass the numerus fixus and secure admission to medical school at Maastricht University in 2009.

During the first two years of medical school, Mark ranked among the top 3% of students at the Faculty of Health, Medicine and Life Sciences. His enthusiasm for research further developed around 2010 at the Department of Immunology and Nephrology, where he also completed the honours research program in 2011. Clinical rotations, including electives at Manipal University in India, Tygerberg Hospital, affiliated with Stellenbosch University, in South Africa, and Atrium MC (now Zuyderland MC) in Heerlen, confirmed his preference for pediatric medicine.

Mark graduated in Medicine in 2015 and subsequently worked as a medical doctor not in training ('ANIOS') at the Department of Pediatrics at Catharina Hospital Eindhoven (2015–2016) and at Maastricht UMC+ (2016–2017). In 2017, he was accepted into the pediatric residency program at Maastricht UMC+ and at Máxima Medical Centre in Veldhoven (2018–2020). From the start of his training, his career goal was clear: to become a pediatric nephrologist. He completed the International Pediatric Nephrology Association (IPNA) - European Society for Paediatric Nephrology (ESPN) Master for Junior Classes in 2021 and attended several IPNA-ESPN conferences, delivering his first oral presentation at the 2017 ESPN conference in Glasgow, United Kingdom.

In 2021, Mark undertook clinical training at the Department of Pediatric Nephrology at Radboud University Medical Centre Amalia Children's Hospital in Nijmegen, which marked the beginning of his fellowship in this speciality. He became a pediatrician in March 2023 and obtained certification as a pediatric nephrologist in February 2025. He is currently working in MUMC+ MosaKids Children's Hospital as a pediatric nephrologist. In addition, he is a member of the steering committee of the Kidnie Research Consortium on behalf of MUMC+ MosaKids Children's Hospital.

Mark is married to Marisya van Dam - Janssen, and together they are the proud parents of their daughters, Elea and Nine (†).

LIST OF PUBLICATIONS

van Dam M, Rijks J, Dorenbos E, Horuz F, van Dael K, Vreugdenhil A. The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity. *Sci Rep*. 2019 Mar 14;9(1):4504

Timmermans SAMEG*, **van Dam MJCM***, Vink E, Horuz FAPT, van Paassen P, Rosias PPR. Rituximab for the Treatment of Pediatric Double-Positive Small-Vessel Vasculitis. *Kidney Int Rep*. 2019 Nov 26;5(2):235-238 * shared first author

van Dam MJCM, Zegers BSHJ, Schreuder MF. Case Report: Uterine Anomalies in Girls With a Congenital Solitary Functioning Kidney. *Front Pediatr*. 2021 Dec 14;9:791499

van Dam MJCM, Pottel H, Vreugdenhil ACE. Creatinine-based GFR-estimating equations in children with overweight and obesity. *Pediatr Nephrol*. 2022 Oct;37(10):2393-2403

van Dam MJCM, Pottel H, Vreugdenhil ACE. Relation between obesity-related comorbidities and kidney function estimation in children. *Pediatr Nephrol*. 2023 Jun;38(6):1867-1876

van Dam MJCM, Pottel H, Delanaye P, Vreugdenhil ACE. The evaluation of kidney function estimation during lifestyle intervention in children with overweight and obesity. *Pediatr Nephrol*. 2024 Nov;39(11):3271-3278

