Correlates of Leptin in Children with Chronic Kidney Disease

Edward Nehus, MD[1], Susan Furth, MD, PhD[2], Bradley Warady, MD[3], and Mark Mitsnefes, MD[1]

Objective To investigate the relative associations of renal function, obesity, and inflammation with serum leptin levels in children with chronic kidney disease (CKD).

Study design This was a cross-sectional analysis of 317 children from the Chronic Kidney Disease in Children study, a large cohort of pediatric patients with stage II-IV CKD. Linear regression modeling was used to evaluate the association of serum leptin level with glomerular filtration rate calculated using the plasma iohexol disappearance curve, demographics, body mass index (BMI), and cardiovascular risk factors, including inflammatory cytokines, insulin resistance, and serum lipid levels.

Results In univariate analyses, elevated serum leptin level was significantly associated with increased BMI, older age, and female sex ($P < .001$ for all). Leptin level also correlated positively with serum triglycerides and insulin resistance ($P < .001$) and negatively with serum high-density lipoprotein cholesterol ($P = .002$). Leptin level was not associated with glomerular filtration rate calculated using the plasma iohexol disappearance curve or inflammatory cytokines. In multivariate analysis, BMI, age, female sex, and serum triglyceride levels were significantly associated with serum leptin level.

Conclusion Increased leptin production was associated with female sex, older age, and adiposity in children with mild to moderate CKD. Renal function was not associated with serum leptin level, indicating that decreased clearance does not contribute to elevated leptin levels. *(J Pediatr 2014;165:825-9)*.

Leptin is a hormone produced by adipocytes that acts centrally to signal satiety and increase energy expenditure. The association of leptin with adiposity has been interpreted as a state of "leptin resistance" that may be involved in the pathogenesis of obesity.[1-3] Furthermore, the association of leptin with inflammation, insulin resistance, and hyperlipidemia has suggested a role for leptin as a mediator of cardiovascular disease.[4-8]

Leptin levels are elevated in patients with advanced chronic kidney disease (CKD) and may be involved in the inflammation, malnutrition, and cardiovascular disease that occur in this population.[9,10] The putative cause of increased leptin levels in CKD has not been determined. Although leptin is a 16-kDa molecule excreted by the kidney,[11,12] the relative contribution of decreased renal clearance to total serum leptin level is unknown. Increased production in association with increased fat stores and inflammation are other potential causes of hyperleptinemia in CKD.[13-16]

The objective of this study was to investigate the relative associations of renal function, obesity, and inflammation with leptin levels in children with CKD. We performed a cross-sectional analysis of 317 children enrolled in the Chronic Kidney Disease in Children (CKiD) study, a prospective observational study of children with mild to moderate CKD. We hypothesized that leptin was primarily associated with body mass index (BMI) and renal function in children with CKD.

Methods

The CKiD study is a multicenter, longitudinal, observational study of children with CKD whose design and methods have been published previously.[17] Institutional Review Board approval was obtained at each participating center. Goals of the CKiD study include evaluating novel and traditional risk factors for cardiovascular disease in children with CKD. Inclusion criteria were age 1-16 years and a glomerular filtration rate (GFR) of 30-90 mL/min/1.73 m^2.

The present study is a cross-sectional analysis of baseline serum leptin levels obtained in the initial CKiD cohort at year 2 of the study. The initial CKiD cohort

BMI	Body mass index
CKD	Chronic kidney disease
CKiD	Chronic Kidney Disease in Children study
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
iGFR	Glomerular filtration rate calculated using plasma iohexol disappearance curve
IL	Interleukin

From the [1]Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Division of Nephrology, The Children's Hospital of Philadelphia, Philadelphia, PA; and [3]Division of Pediatric Nephrology, Children's Mercy Hospital, Kansas City, MO

Funded by the National Institute of Diabetes and Digestive and Kidney Diseases (DK076957 to M.M). The CKiD Study is supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, and the National Heart, Lung, and Blood Institute (U01-DK-66143, U01-DK-66174, U01DK-082194, U01-DK-66116). The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2014.06.030

comprised 586 children at 46 pediatric nephrology centers in North America. Of these study participants, 317 had serum available for analysis of leptin level.

Serum leptin analysis was performed at the Metabolic Phenotyping Core, University of Texas Southwestern Medical Center, Dallas, using serum samples from the CKiD study. Original samples were collected locally, shipped, and stored at the National Institute of Diabetes and Digestive and Kidney Diseases' Biologic Repository at −80°C. Serum leptin levels were determined using the Human Leptin ELISA Kit (Millipore, Billerica, Massachusetts).

Fasting insulin levels (Human Insulin ELISA; Millipore), tumor necrosis factor-α, interleukin (IL)-6, and IL-10 from the same sample were determined by the same laboratory. A multiplex bead array (Bio-Rad, Hercules, California) using a Luminex 100 system (Luminex, Austin, Texas) was used to determine cytokine concentrations. This assay has been validated and has demonstrated excellent linearity, precision, and sensitivity.[18-20] The homeostasis model assessment of insulin resistance (HOMA-IR), calculated by dividing the product of serum insulin (mU/mL) and glucose (mmol/mL) by a factor of 22.5, served as a measure of insulin resistance.

Other laboratory data obtained for analysis included fasting lipid profiles (high-density lipoprotein [HDL] cholesterol, low-density lipoprotein cholesterol, and triglycerides) and urine protein to creatinine ratios. GFR was calculated using the plasma iohexol disappearance curve (iGFR), as described previously.[17] Demographic data were obtained concurrent with serum leptin levels and other laboratory data and included age, sex, Tanner stage, casual blood pressure, and BMI. Children were classified as overweight or obese as recommended by the Centers for Disease Control and Prevention.[21] Overweight was defined as an age- and sex-specific BMI at or above the 85th percentile and lower than the 95th percentile, and obese as an age- and sex-specific BMI at or above the 95th percentile.

Statistical Analyses

Continuous variables are reported as median and IQR, because most variables were non-normally distributed. Nonparametric testing was used for categorical variables (Wilcoxon rank-sum test or Kruskal-Wallis test). Pearson correlation coefficients were used for continuous independent variables after log transformation as needed to fulfill the assumptions of linear regression modeling. Laboratory values that were log-transformed included serum leptin, HOMA-IR, serum insulin, inflammatory markers, and lipid profiles. Multivariable regression analysis was performed using all variables with a P value of <.10 in univariate analyses. Backward elimination, selecting variables significant at the .05 level, was then performed for the final regression model. All analyses were conducted using SAS 9.3 (SAS Institute, Cary, North Carolina).

Results

Baseline characteristics of the cohort are presented in Table I. Fifteen percent of children were obese. Approximately one-half were prepubertal, with the remainder distributed among all 4 stages of pubertal development. Most children had a nonglomerular cause of CKD. The most common etiologies were obstructive uropathy, renal dysplasia, and reflux nephropathy (21%, 17%, and 16% of the entire cohort, respectively). Focal segmental glomerulosclerosis and hemolytic uremic syndrome were the most prevalent glomerular etiologies, occurring in 7% and 4% of the cohort, respectively.

Table I. Baseline characteristics of the CKiD cohort (n = 317)

Characteristics	Value
Age, y, median (IQR)	12.4 (8.9-15.6)
Sex, %	
Male	57
Female	43
Race, %	
Caucasian	72
African-American	13
Other	15
Cause of CKD, %	
Glomerular	21
Nonglomerular	79
Tanner stage, %	
I	48
II	12
III	9
IV	13
V	17
Duration of CKD, y, median (IQR)	7.6 (4.3-11.7)
iGFR (mL/min/1.73 m^2), median (IQR)	45 (34-58)
Height percentile, median (IQR)	25 (8-51)
Weight percentile, median (IQR)	45 (21-77)
BMI percentile, median (IQR)	59 (33-87)
Systolic blood pressure percentile, median (IQR)	59 (29-82)
Diastolic blood pressure percentile, median (IQR)	61 (39-85)
HDL, mg/dL, median (IQR)	48 (41-56)
LDL, mg/dL, median (IQR)	104 (84-121)
Triglycerides, mg/dL, median (IQR)	107 (74-143)
IL-6, pg/mL, median (IQR)	1.9 (1.1-3.5)
IL-10, pg/mL, median (IQR)	2.3 (1.6-4.3)
TNF-α, pg/mL, median (IQR)	5.0 (3.6-8.2)
Leptin, ng/mL, median (IQR)	3.4 (1.5-15.5)
Glucose, mg/dL, median (IQR)	89 (83-95)
Insulin, mU/L, median (IQR)	7.6 (4.2-12.2)
HOMA-IR, median (IQR)	1.7 (0.9-2.8)
Protein-to-creatinine ratio, median (IQR)	0.44 (0.18-1.1)

LDL, low-density lipoprotein; *TNF*, tumor necrosis factor.

Serum Leptin Levels and Patient Demographics

Serum leptin levels did not differ by race. Median levels were higher in children with a glomerular etiology of CKD compared with children with a nonglomerular cause (7.1 ng/mL vs 2.9 ng/mL; $P < .01$). Median leptin levels were also higher in females than in males (6.9 ng/mL vs 2.4 ng/mL; $P < .001$).

Leptin levels by Tanner stage are displayed in Table II. Leptin levels increased throughout puberty in females, whereas males showed a mild increase in early puberty with no subsequent rise. Leptin levels were significantly higher in obese children compared with nonobese children (median, 33.2 ng/mL vs 3.0 ng/mL; $P < .001$) (Figure 1).