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Abstract

Plasma prekallikrein (PK) has been shown to be associated with cardiovascular disease (CVD) and its risk factors, but these associations have not been investigated in children. The present study examined PK activity in relation to well-established cardiovascular risk factors in a cohort of children aged 9–11 years ($N = 97$). We found a significant and positive association between PK and fasting levels of total cholesterol ($p < 0.01$), non-high-density lipoprotein cholesterol ($p < 0.01$), and triglycerides ($p < 0.001$). In addition, there was a significant association between PK activity and the metabolic syndrome, a clustering of risk factors considered to have an impact on atherosclerosis and CVD mortality. Finally, we found that children with a family history of CVD had significantly elevated PK activity. These novel findings warrant further investigations into the relationship between circulating PK levels and CVD risk factors because PK may be involved in the progression of the disease state.

Keywords*: prekallikrein, plasma, lipids, cardiovascular disease, metabolic syndrome, children.

Résumé

Il est bien connu que la pre´kallicre´ine (PK) est associe´e a` la maladie cardiovasculaire (CVD) et a` ses facteurs de risque, mais ces associations n'ont pas e´te´ ve´rifie´es chez les enfants. Cette e´tude se propose de ve´rifier l'association entre l'activite´ de la PK et les facteurs de risque de CVD aupre`s d'une cohorte d'enfants aˆge´s de 9 a` 11 ans ($N = 97$). On observe une corre´lation positive significative entre la PK et les variables suivantes : la concentration totale de choleste´rol a` jeun ($p < 0,01$), les concentrations des lipoprote´ines non a` haute densite´ ($p < 0,01$) et la concentration des triglyce´rides ($p < 0,001$). De plus, on observe une corre´lation significative entre l'activite´ de la PK et le syndrome me´tabolique constitue´ par un ensemble de facteurs ayant un effet sur l'athe´roscle´rose et la mortalite´ due a` la CVD. En dernier lieu, les enfants avec des ante´ce´dents familiaux de CVD pre´sentent une plus importante activite´ de la PK, et ce, de fac¸on significative. Ces premie`res observations sugge`rent d'autres e´tudes sur la relation entre la concentration sanguine de PK et les facteurs de risque de CVD, car la PK pourrait eˆtre implique´e dans la progression de la maladie.

Mots-cle´s *: pre´kallicre´ine, plasma, lipides, maladie cardiovasculaire, syndrome me´tabolique, enfants.
[Traduit par la Re´daction]

1. Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in the United States and disables 10 million Americans each year (CDC 2004). Studies involving children and young adults have shown that cardiovascular risk factors are present very early in life, suggesting that efforts to prevent CVD should focus on targeting the childhood origins of the disease. For example, asymptomatic children and young adults who were autopsied following accidental death, homicide, or suicide were found to have the beginning stages of atherosclerosis, which significantly correlated with increasing cardiovascular risk factors (Berenson et al. 1998; McGill et al. 2000). CVD risk factors are not only present in childhood, but are also predictive of future CVD. Coronary artery calcification measured in 29- to 37-yearolds was worse in those who had an increased body mass index (BMI) in childhood and young adulthood and elevated blood pressure and low high-density lipoprotein cholesterol (HDL-C) in young adulthood (Mahoney et al. 1996). Furthermore, childhood low-density lipoprotein cholesterol (LDL-C) levels, systolic blood pressure (SBP), and childhood BMI were found to be associated with greater carotid intima-media thickness in adulthood (Li et al. 2003; Raitakari et al. 2003). Establishing the important CVD risk factors in childhood becomes even more critical as today's children are becoming less healthy; one third of American children

and adolescents are obese or overweight and this population is more likely to possess other CVD risk factors as well (Daniels et al. 2009).

One important cardiovascular and metabolic risk is the metabolic syndrome (MetS), a constellation of risk factors that includes abdominal obesity, dyslipidemia, raised blood pressure, insulin resistance (with or without glucose intolerance), prothrombotic state, and proinflammatory state (NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002). The MetS is associated with an increased CVD mortality in adults (Ford 2004; Saito et al. 2009), and a recent study has shown that MetS risk factors are also present in children. Analysis of data from the National Health and Nutrition Examination Survey (2001– 2006) found that the prevalence of the MetS in adolescents aged 12–19 years was 8.6% (Johnson et al. 2009).

High levels of circulating triglycerides (TG) and low levels of HDL-C are 2 of the 5 criteria defining the MetS in children (de Ferranti et al. 2004) and constitute important risk factors for CVD (NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002; Le and Walter 2007). A lipid profile of low HDL-C, high LDL-C, high total cholesterol (TC), and elevated TG is an important factor in coronary calcification, a measure of the atherosclerotic process (McCullough 2005). Non-HDL C, which comprises all atherogenic apo-B-containing lipoproteins, was reported recently as being more strongly associated with subclinical atherosclerosis than were all other conventional lipid values, including LDL-C, HDL-C, and TG (Orakzai et al. 2009).

In cross-sectional studies, the plasma kallikrein–kinin system (KKS) has been shown to be associated with CVD and its risk factors, including circulating lipids. The KKS is involved in inflammation, blood pressure control, coagulation, and pain, and consists of the blood proteins prekallikrein (PK), factor XII, and high-molecular-weight kininogen (HK) (Sainz et al. 2007; Schmaier 2008). Plasma PK is activated to form kallikrein, which cleaves HK to liberate the vasoactive peptide bradykinin, a potent stimulator of endothelial cell prostacyclin synthesis, tissue plasminogen activator release, and the formation of superoxide, nitric oxide, and smooth muscle hyperpolarization factor (Schmaier 2008). In adults, elevated cholesterol has been reported to be associated with decreased blood PK levels (Carvalho et al. 1978), and cholesterol-lowering drugs cause an increase in HDL-C with an associated rise in PK (Torstila et al. 1982). Elevated plasma PK levels have been found to be associated with an increased risk of myocardial infarction (Merlo et al. 2002). Plasma PK levels are also higher in diabetic and hypertensive rats, which have higher mean arterial pressure and left ventricular wall mass (Sharma and Kesavarao 2007). Although these cross-sectional studies clearly suggest an association between PK and CVD, we are unaware of any prospective cohort studies that have looked at the ability of PK to be predictive of future CVD. This study used a cross-sectional cohort of 97 children aged 9–11 years to investigate the hypothesis that circulating plasma PK are associated with known CVD risk factors at an early age.

2. Materials and methods

2.1 Study population

Participants were recruited for an ongoing study of the effects of heavy metals on cardiovascular function. Of the 140 children enrolled in the Oswego Lead Study, we included 97 children (55 boys and 42 girls) in the present analyses. Children were excluded if they were diabetic ($n = 2$), or if we were unable to obtain a resting blood pressure ($n = 1$), or if blood draws were not concurrent with the anthropomorphic data collection ($n = 40$). Children were 9–11 years old and their families were paid US$100 for their participation. The study was approved by the Institutional Review Board of the State University of New York College at Oswego (Oswego, N.Y.). Each child and parent gave written assent and consent, respectively.

2.2 Biochemical parameters

Fasting blood samples were collected in the morning. Serum was collected in a 4-mL Griener Vacuette Serum Gel Evacuated Tube (Greiner Bio One North America Inc., Monroe, N.C.) and shipped immediately to the Oswego Hospital Laboratory (Oswego, N.Y.). Glucose, TG, TC, and HDL-C were all analyzed on the Siemens Advia 1800 chemistry analyzer (Siemens Healthcare Diagnostics Inc., Deerfield, Ill.). LDL-C was calculated using the Friedewald equation: LDL-C = (TC – HDL-C) – (TG _ 0.20). Non-HDL-C was calculated as TC – HDL-C (NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002). Plasma was collected in a 4-mL Greiner Vacuette K3 EDTA Evacuated Tube (Greiner Bio One North America Inc.) and prepared for storage within 1 h of being drawn. Plasma was double spun to remove all cellular contaminants, aliquoted to reduce freeze-thaws, and stored at –80°C until used for PK analysis. To activate plasma PK, 18.2 µL of 25 mg_L^{-1} dextran sulfate (Sigma–Aldrich, St. Louis, Mo.) in water was added to an equal volume of plasma and incubated on ice for 7 min (Kluft 1978). Kallikrein activity was then detected by hydrolysis of chromogenic substrate H-D-Pro-Phe-Argparanitroanilide (S-2302) (DiaPharma, Franklin, Ohio) (De La Cadena et

al. 1987). Activated plasma samples were diluted to 200 µL with cooled 50 mmol_L^{-1} Tris–HCl buffer, pH 7.8, warmed to 37°C, mixed with an equal volume of prewarmed S-2302, and incubated for 2 min at 37°C. The reaction was stopped by the addition of 200 µL of 50% acetic acid, and the optical density of the samples was read at 405 nm. PK values were expressed as U-L^{-1} and were calculated using the extinction coefficient of 10 600 at A$_{405}$ for p-nitroanilide (De La Cadena et al. 1987). Pooled human plasma was used as an internal control. The intra-assay and inter-assay coefficients of variance were 3.0% and 5.7%, respectively.

2.3 Clinical evaluation

Within 1 week of the blood draw, medical and family histories were completed by a parent, and the child's anthropomorphic measures were taken by technicians trained using the videotaped procedures outlined by the Centers for Disease Control and Prevention–National Center for Health Statistics for the Third National Health and Nutrition Examination Survey (CDC–NHANES 1996). Children's height (in centimeters) and mass (in kilograms) were used to calculate age-adjusted and gender-adjusted BMI percentile scores using a SAS script developed by the Centers for Disease Control and Prevention (CDC 2007). SBP and dia stolic blood pressure were monitored using a Vasotrac device (APM 205A, Medwave, Danvers, Mass.), which is a noninvasive, radial artery tonometry device that has been validated against simultaneous indwelling radial arterial catheters (Thomas et al. 2004). The Vasotrac sensor was positioned on the nondominant wrist and measurements in mmHg were recorded every 30 s during the final 3 min of a 10-min rest. Blood pressure percentiles for age, gender, and height were calculated as recommended by the National Heart, Lung, and Blood Institute (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2004). The MetS was defined as having ≥3 of the following: (*i*) fasting ≥100 mg-dL^{-1}, (*ii*) HDL < 50 mg-dL^{-1}, (*iii*) fasting glucose ≥110 mg_dL^{-1}, (*iv*) waist circumference (WC) ≥ 90th percentile, and (*v*) SBP ≥ 90th percentile for gender, age, and height (de Ferranti et al. 2004). We chose to use a ≤90th percentile cut-off for WC, rather than the original 75th percentile that de Ferranti used, because the 90th percentile is the most commonly used threshold for WC (Ford and Li 2008). WC percentile cut-offs for gender and age were used as previously described (Ferna´ndez et al. 2004).

2.4 Statistical analysis

The Gaussian distribution of each data set was examined using the Kolmogorov–Smirnov test in Prism 4.03 (GraphPad Software Inc., La Jolla, Calif.). Those that were found to be not normally distributed (TG, TC, HDL-C, and WC) were log transformed for all univariate regression analyses. All other analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill.). Univariate regression analyses were performed with kallikrein serving as the dependent variable. A general linear model was used to analyze PK levels in relation to MetS status (defined as 0 for no and 1 for yes) and the number of MetS risk factors (defined as 0 for none, 1 for any 1 factor, 2 for any 2, and 3 for 3 or more factors). A planned linear contrast tested whether PK levels increased linearly across these groups. Odds ratios were calculated using Fisher's exact test to estimate the relative risk of having a cluster of non-TG MetS risk factors (defined as 0 for having 0 or 1 risk factor and 1 for having 2 or more risk factors) for circulating levels of either PK or TG. The risk of possessing the clustered non-TG risk factors (as defined above) based on a combination of PK and TG levels was estimated after separating subjects into 4 groups: normal levels of both PK and TG, elevated PK only, elevated TG only, and elevated levels of both PK and TG. Fisher's exact test was used with the group having normal PK and TG serving as the reference group. Student's t test was used to determine the statistical significance of family histories. For all analyses, p values were 2 sided and were considered significant if ≤0.05. The effect of possible confounders (age, gender, race, and socioeconomical status) on these associations was tested individually and together, and there was no significant change in the size of any of the correlations when these variables were added as covariates (data not shown).

3. Results

3.1 Subject characterisitcs

Table 1 shows the characteristics of the study population, which consisted of 97 children aged 9, 10, and 11 years ($N = 43$, $N = 49$, and $N = 5$, respectively) that were free of known CVD or diabetes. There were roughly an equal number of males and females, and the population was predominantly white (90%). The gender-adjusted and age-adjusted mean BMI percentile was 74.9; 27.8% were obese, having a BMI ≥95th percentile. The mean PK level was 1656.8 U-L^{-1} and there was no statistical difference between males and females (t (95) = 1.33, $p > 0.05$).

3.2 PK and individual CVD risk factors

Plasma PK activity is positively associated with an unhealthy plasma lipid profile. Univariate regression analysis showed that TC ($F_{[1.95]}$ = 7.56, $p < 0.01$), non-HDL-C ($F_{[1.95]}$ = 8.89, $p < 0.01$), and TG ($F_{[1.95]}$ = 26.42, $p < 0.001$)

were significantly and positively correlated with PK levels (Table 2). There was no significant relationship found between PK levels and resting blood pressure, BMI, WC, or fasting levels of HDL-C, LDL-C, and glucose (all p values >0.05).

3.3 PK and the MetS

An increase in the number of MetS risk factors is associated with increased PK levels when using risk factor cut-off points established for a pediatric population (de Ferranti et al. 2004; Ford and Li 2008). The prevalence of each MetS risk factor was as follows: 25.8% had an increased WC, 3.1% were hyperglycemic, 15.5% were hypertensive, 18.6% had elevated TG levels, and 34% had low HDL-C levels. Of the children who met 3 of the 5 criteria and therefore qualified as having the MetS (8.2%), the only risk factor shared by all of them was WC. Mean PK levels were significantly higher in children with the MetS (1928.5 ± 340.9 U·L^{-1}), compared with those without (1632.3 ± 306.1U·L^{-1}) (t (95) = 2.60, $p < 0.05$; Fig. 1A). We also determined the relationship between PK and the number of risk factors the child possessed, which were as follows: none (41.2%), any 1 (30.9%), any 2 (19.6%), any 3 or 4 (8.2%). Only 1 child had 4 of the criteria and none had all 5. When children were grouped in this manner, there was a significant linear trend of increasing mean PK levels with an increasing number of MetS risk factors (p for linear trend <0.05; Fig. 1B).

Because TG levels are among the 5 criteria for the MetS, and PK levels were related to the MetS, we next considered whether these 2 variables act synergistically, placing children at a significantly elevated risk for the MetS when both are elevated. Table 3 shows the odds ratio (OR) of having 2 or more of the non-TG MetS risk factors for different combinations of PK and TG levels. TG cut-off points were used as before and, because there are no established guidelines for high PK levels, we considered high to be the upper quartile (75% = 1840.4 U·L^{-1}). When considered individually, children with high PK levels did not have a significantly different risk of having 2 or more of the non-TG MetS risk factors, compared with those with low PK levels (OR 2.1, 95% CI 0.67–6.57), and those with high TG levels did not have a significantly different risk (OR 2.4, 95% CI 0.71–7.99). As such, having either high TG or PK levels alone did not significantly increase the risk of the MetS; however, when children had both high TG and PK levels, they had a 4.9 times greater risk (1.10–21.74).

Table 1. Characteristics of study population (N = 97).

Variable	Mean±SD	%	No. (%) that meet MetS cut-off
Age (y)	10.0±0.6		
Gender (% female)		43.3	
Race (% white)		90.0	
BMI (percentile)		74.9	
PK (U·L^{-1})	1656.8±317.9		
SBP (mm Hg)	104.6±11.1		15 (15.5)
DBP (mm Hg)	56.0±7.1		—
WC (cm)	69.6±10.2		25 (25.8)
TC (mg·dL^{-1})	164.7±29.2		—
HDL-C (mg·dL^{-1})	55.2±12.4		33 (34.0)
Non-HDL-C (mg·dL^{-1})	109.6±26.5		—
LDL-C (mg·dL^{-1})	95.3±24.0		—
TG (mg·dL^{-1})	71.0±37.5		18 (18.6)
Glucose (mg·dL^{-1})	95.9±6.5		3 (3.1)

Note: Cut-offs for MetS criteria are as described in Materials and methods section. MetS, metabolic syndrome; BMI, body mass index; PK, prekallikrein; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

Table 2. Regression analysis for plasma prekallikrein and cardiovascular risk factors.

Risk factor	Correlation coefficient	p
SBP (percentile)	0.01	0.888
DBP (percentile)	0.02	0.840
BMI (percentile)	0.10	0.315
WC (log cm)	0.11	0.275
TC (log mg·dL^{-1})	0.27	0.008*
HDL-C (log-mg·dL^{-1})	–0.02	0.880
Non-HDL-C (mg·dL^{-1})	0.29	0.004*
LDL-C (mg·dL^{-1})	0.18	0.074
TG (log mg·dL^{-1})	0.47	<0.001*
Glucose (mg·dL^{-1})	–0.09	0.409

Note: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.
*Statistically significant.

3.4 PK and family history of CVD

Plasma PK levels are higher in children who have a family history of CVD (Fig. 2). Having a family history of CVD (81.4%) was based on having at least 1 parent or grandparent who had been given a diagnosis of either hypertension (73.1%), heart disease (49.5%), or stroke (19.6%). There was a significant increase in the PK levels of children with a family history of CVD (1509.0 ± 291.8 U·L^{-1}) compared with those without (1687.3 ± 318.1 U·L^{-1}) (t (95) = 2.45, $p < 0.05$). This difference was not seen for other diseases that are also known to have an inheritable component, including asthma (30.9% reporting a family history), diabetes (36.1%), and cancer (51.5%) (all $p > 0.50$).

4. Discussion

Studies suggest that tissue and plasma PK–kallikrein play a role in cardiovascular regulation and dysfunction. At the tissue level, renal PK–kallikrein, as measured through urinary kallikrein activity, is lower in children with higher blood pressures (Zinner et al. 1976) and in men with at least one parent with a history of hypertension (Lin et al. 1984). Increasing heart kallikrein levels in diabetic rats restores some cardiac function and improves the lipid profile and glucose utilization (Montanari et al. 2005). These studies suggest a protective role of tissue PK–kallikrein. Conversely, some studies suggest that increased plasma PK–kallikrein may be a CVD risk factor. The plasma KKS is composed of a group of proteins that exert their influence on the vasculature through bradykinin, which is liberated from HK by active kallikrein (Sainz et al. 2007; Schmaier 2008). Plasma PK levels are increased in diabetics(Christe et al. 1984; Jaffa et al. 2003) and in rats that are either diabetic or hypertensive (Sharma and Kesavarao 2007). Elevated PK is also associated with an increased risk of myocardial infarction (Merlo et al. 2002) and an increased risk of hypertension in diabetics (Jaffa et al. 2003; Kedzierska et al. 2005). In our study of 97 children aged 9–11 years, we found that increased plasma PK levels were associated with CVD risk factors.

The mean plasma PK level of the children in this study (1657 ± 318 U·L^{-1}) is within the range of 990 (Andrew 1992) to 1660 U·L^{-1} (Kallen and Lee 1975) that has been described previously in children. PK levels were significantly and positively associated with fasting levels of TG, TC, and non-HDL-C but were not associated with glucose, HDL-C, or LDL-C. These observed PK–lipid relationships are contradictory to studies in adults.

Hypercholesterolemia patients were reported to have decreased PK levels compared with controls, and patients with hypertriglyceridemia had no differences in PK relative to controls (Carvalho et al. 1978). Additionally, treating hypercholesterolemia patients with gemfibrozil caused a significant rise in PK and an associated increase in HDL-C (Torstila et al. 1982).

Fig. 1. Mean levels of prekallikrein (PK) are higher in children with the metabolic syndrome (MetS). (A) PK levels of children with the MetS (defined as possessing ≥3 criteria) or without the MetS (<3 criteria). (B) PK levels by the number of MetS risk factors present. The numbers of subjects possessing MetS risk factors were as follows: none, 40; any 1, 30; any 2, 18; any 3 or 4, 8.
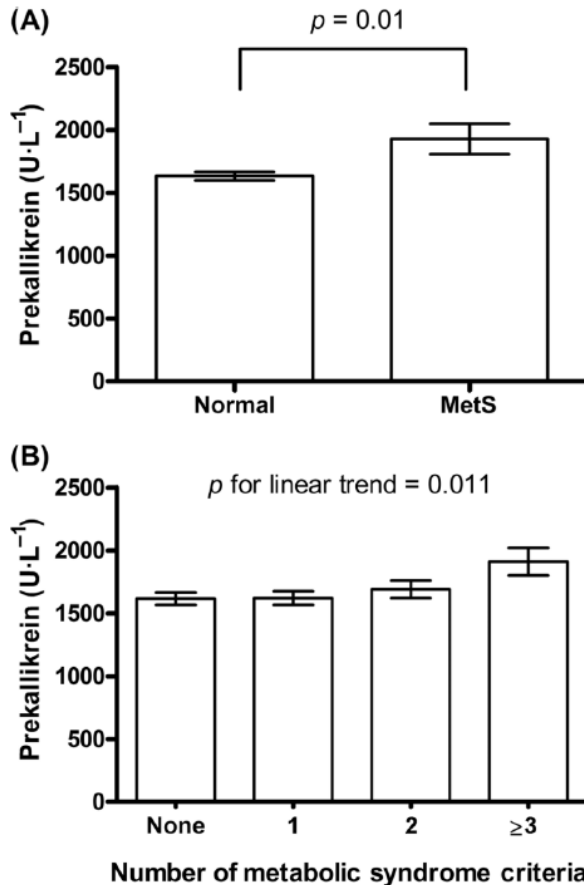
Fig. 2. Prekallikrein levels are elevated in children with a family history of cardiovascular disease (CVD). The numbers of subjects identifying as having a family history were as follows: CVD, 79; asthma, 30; diabetes, 35; and cancer, 50.
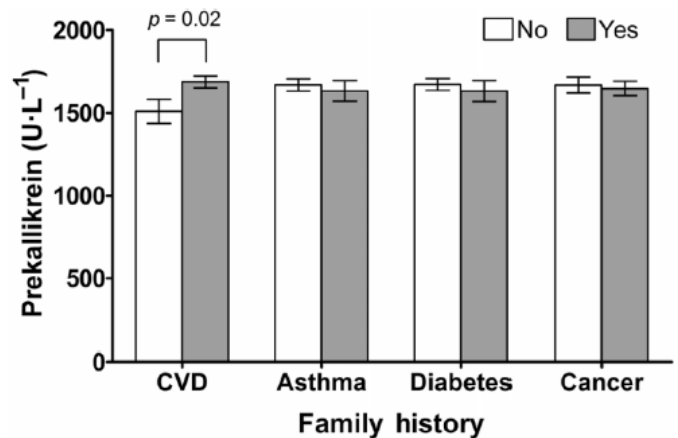
Table 3. Odds ratio (OR) of possessing 2 or more of the nontriglyceride metabolic syndrome risk factors by levels of prekallikrein and triglycerides alone and in combination with one another.

	Level	OR	95% CI
Considered individually			
Prekallikrein	Low	1.00	
	High*	2.10	0.67–6.57
Triglycerides	Low	1.00	
	High†	2.38	0.71–7.99
Considered together			
Prekallikrein–triglycerides	Low–low	1.00	
	High*–low	0.94	0.18–4.88
	Low–high†	0.76	0.09–6.86
	High*–high†	4.89	1.10–21.74

Note: CI, confidence interval.
*High prekallikrein is defined as ≥75 percentile.
†High triglycerides is defined as fasting levels >100 mg·dL^{-1}. ORs were calculated as compared with having low prekallikrein and triglyceride levels alone or combined.

The inverse relationship between PK and a healthy lipid profile has been suggested to result from enhanced removal of plasma PK from the circulation as it is activated to kallikrein during intravascular coagulation (Carvalho et al. 1978). This is supported by the finding that circulating lipoproteins are able to activate plasma PK (Larsen et al. 2000). However, a review of the literature does not reveal any studies that have described a mechanism for maintaining homeostatic control over circulating plasma PK levels. Such a mechanism likely results from activation in tissues and (or) synthesis by the liver. Plasma PK is activated in the vasculature by prolylcarboxypeptidase (PRCP), which is constitutively expressed on endothelial cell membranes, and the recombinant PRCP activates PK with a Km ∼ 9–17 nmol_L–1 (Schmaier 2008). Because the regulation of PRCP expression and degradation is unknown (Mallela et al. 2009), we cannot comment on the possible role that PRCP plays in the relationship between circulating PK and lipids. We hypothesize that PK is related to an unhealthy lipid profile (primarily high TG and non-HDL-C) because of an increase in hepatic protein synthesis, as patients with hypercholesterolemia and hypertriglyceridemia have an increase in hepatic protein synthesis, including proteins of the coagulation system (Brudaşca̧ and Cucuianu 2007).

PK levels of the children in this study were higher for those with risk factors, which include high TG, low HDL-C, high glucose, elevated WC, and elevated SBP, that define the pediatric MetS (Ford and Li 2008). The linear relationship between PK levels and the number of MetS criteria supports the existence of a true association. Univariate regression analysis of the individual components found that the only significant association was the aforementioned one with TG. Neither resting SBP nor WC was related to PK, which contrasts with what has been observed in diabetics, where PK was positively associated with both blood pressure and BMI, a surrogate for WC (Jaffa et al. 2003). We also considered whether TG and PK would work synergistically and increase the risk of having 2 or more of the non- TG MetS factors. Independently from each other, elevated PK and TG levels did not significantly increase the risk of having a cluster of the other risk factors; however, the combination of high PK and TG levels was associated with a significant 4.9-fold risk of having a cluster of the other risk factors.

The reason why PK was not associated with the individual risk factors except for TG, but was associated with the clustered MetS risk factors, could be the age of our subjects. It would be expected that CVD risk factors become more apparent as the child ages and the disease state progresses. A recent study of adolescents found prehypertension to be predictive of future hypertension (Falkner et al. 2008). It is also likely that the appearance of risk factors would differ among individuals and that the risk factors would manifest themselves at different rates. Three of the 5 MetS criteria, namely TG (this study), BMI, and SBP (Jaffa et al. 2003), have now been shown to be positively associated with plasma PK levels. If future studies support that elevated PK levels are a CVD risk factor, we predict that some of our study subjects will begin to display these other risk factors unless there is some change in lifestyle. It is of interest to note that the only risk factor shared by all of the children who qualified as having the MetS was that they all had a WC that was greater than the 90th percentile for their age and gender. This is consistent with the hypothesis that visceral fat accumulation is the primary cause of the MetS (Ryo et al. 2004). It is also in line with the definition of the MetS established by the International Diabetes Federation, which requires the presence of central obesity plus 2 of the other 4 risk factors (Alberti et al. 2006).

Studies investigating renal kallikrein secretion suggest it is a heritable trait. If plasma PK is as well, some people may be predisposed to having elevated PK levels. Urinary kallikrein was found to be higher in children who are black (vs. white) and female (vs. male) (Zinner et al. 1976); however, our data did not find any significant differences between either of these and plasma PK (data not shown). Urinary kallikrein excretion was also shown to be heritable in association with hypertension (Williams et al. 1990) and lower in men who have at least 1 parent with a history of hypertension (Lin et al. 1984). We found that children having a family history of CVD had higher plasma PK levels. The disparity among these findings could be due to differences between renal and plasma PK. Lower urinary kallikrein levels are believed to indicate a diminished paracrine function of the KKS within the kidney. Plasma PK, however, circulates as an inactive proenzyme until it is recruited to a tissue and activated on cell receptors in a multiprotein complex with factor XII and HK (Schmaier 2008). Excess circulating PK would provide a greater likelihood that PK would be found in the tissues where it could become activated tokallikrein.

The current study has several limitations. First, we do not have data on other components of the KKS. However, we predict that factor XII and HK were also elevated, because their levels have been shown to mirror circulating plasma PK levels (Merlo et al. 2002). Second, our sample was relatively small, which can limit generalization to other populations. Even so, the data showed significant associations within this small sample, which suggests the existence of a relatively large effect. Third, accurate generalization to the larger population depends on whether the Oswego Children's Lead Study participants represent the broader population of children. Twenty-eight percent of our participants had a BMI ≥95th percentile, which is higher than the national average of 16.3% for American children and adolescents (Ogden et al. 2008). Even with the higher obesity rate, our participants had the same overall incidence rate of the MetS (8.2%) as was reported previously in adolescents (Johnson et al. 2009), and the incidence rate of the MetS in obese subjects (25.9%) was also similar to national averages (Cook et al. 2003). The nature of our study also limits us from offering any mechanisms by which PK levels can be maintained. Finally, there is some controversy in making the diagnosis of MetS in children younger than 10 years old (Alberti et al. 2006). However, these guidelines suggest that the MetS criteria should be evaluated if there is a family history of the MetS, type 2 diabetes, dyslipidemia, CVD, hypertension, and (or) obesity. Because a recent study found that childhood MetS is predictive of the MetS in adulthood (Morrison et al. 2008), we believe that further investigations into the impact and prevalence of the MetS in children are warranted.

5. Conclusion

To our knowledge, this is the first report to show these PK–lipid associations in children and the PK–MetS associations in children or adults. Additional studies must be conducted before the ramifications of these observations will be known. Our data suggest that PK levels in children are associated with CVD risk factors, especially a poor circulating lipid profile. If PK levels contribute directly to a disease state, then their elevation in

conjunction with lipid abnormalities could act as a tipping point in the progression of the disease state. Further studies relating to plasma PK levels and CVD risk factors are warranted, especially in children, since early life experiences can set a child on a trajectory for increased CVD risk.

6. Acknowledgements

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