Importance of Cystatin C and Uric Acid Levels in the Association of Cardiometabolic Risk Factors in Japanese Junior High School Students

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Abstract

Background: Serum cystatin C (CysC), a novel marker of renal function, is associated with the components of metabolic syndrome in adults. Little is known about the utility of CysC and its association with cardiometabolic risks in young subjects.

Methods and Results: In a cohort of 454 Japanese junior high school students, the distribution of serum CysC levels and associated variables were analyzed. CysC levels were significantly higher in boys than in girls (0.92 ± 0.10 vs. 0.77 ± 0.08 mg/L, $p < 0.001$). CysC was significantly correlated with serum creatinine ($r = 0.473$, $p < 0.001$), and serum uric acid (SUA) ($r = 0.546$, $p < 0.001$). Multivariable regression analysis revealed significant associations between CysC and SUA in all subjects ($\beta = 0.248$, $p < 0.001$), and in boys and girls separately ($\beta = 0.280$ and 0.240, respectively, both $p < 0.001$). Importantly, subjects with elevation of both serum CysC and SUA levels had the highest ratio of triglyceride to high-density lipoprotein cholesterol.

Conclusions: CysC had significant associations with both creatinine and SUA in Japanese junior high school students. The concomitant elevation of serum CysC and SUA levels was associated with subclinical lipid metabolism dysregulation, and suggested the presence of cardiometabolic risk accumulation.

Key Words: Serum uric acid; Cystatin C; Adolescent; Cardiovascular risk

Introduction

Fatty streaks, indicating an early stage of atherosclerosis, have been identified in autopsy samples from children aged <10 years [1-3]. There is an emerging need for effective screening tests that could identify young subjects at high risk for future cardiovascular events. Apart from traditional risk factors, potential biomarkers of subclinical atherosclerosis have been proposed.

Renal dysfunction is associated with the presence of cardiovascular disease and poor outcomes in both children and adults [4]. The screening and early diagnosis of renal dysfunction is, however, not easy in children, because the serum creatinine level does not drop until the glomerular filtration rate (GFR) reaches 30 mL/min or less, and may depend on skeletal muscle mass. Moreover, the equation for estimation of GFR has not been fully established in young adolescents. Sensitive and specific biomarkers for renal function are, therefore, needed to identify high-risk young subjects.

Cystatin C (CysC), a non-glycosylated 13 kDa protein, is sensitive in the detection of early kidney damage [5], because its level may increase when GFR is 70 mL/min or less. CysC levels are reported to be significantly associated with components of metabolic syndrome or cardiovascular diseases in adults. Moreover, high CysC levels are associated with the incidence of major cardiovascular events, including all-cause death, myocardial infarction, cerebrovascular disease and peripheral vascular disease

[6-8], especially in the elderly, and also in subjects without chronic kidney disease [9]. In contrast, little is known about CysC and its clinical significance in young subjects [10]. We previously demonstrated in Japanese junior high school students that the highest quartile of serum uric acid (SUA) was significantly associated with the presence of multiple cardiometabolic risk factors only in boys [11]. A similar gender difference in the association between SUA levels and the incidence of carotid plaque was reported in Japanese adults [12]. To the best of our knowledge, there has been no previous data concerning the association between serum levels of CysC and accumulation of cardiometabolic risks in junior high school students. In the current study, we examined CysC levels in a cohort of Japanese junior high school students and analyzed their clinical utility for health screening in combination with other cardiometabolic risk factors.

Methods

Study Participants and Design

We conducted a cross-sectional survey of health screening in a cohort of 454 Japanese junior high school students (216 boys, 238 girls, age 12.1-15.0 years) between April 2012 and April 2014. The study was a part of the Study Project on Prevention of Metabolic Syndrome among Children, Adolescents, and Young Adults in Shinshu, which was approved by the Medical Ethics Committee of Shinshu University School of

Medicine. Students whose parents gave written informed consent were enrolled, and data were collected from an annual health examination at 3 junior high schools in Nagano prefecture in central Japan.

Measurements

Body height, weight, waist circumference, waist-to-height ratio and body mass index (BMI) were recorded. Systolic and diastolic blood pressure were measured after 2-minutes' seated rest. Waist circumference was measured at the height of the umbilicus at the end of exhalation in the upright position. Venous blood samples after overnight fasting were tested for serum levels of CysC, blood urea nitrogen (BUN), creatinine, SUA and common lipid levels, including triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). The ratio of TG to HDL-C (TG/HDL-C ratio) was also calculated. CysC level was measured using a particle-enhanced immune nephelometric assay. The updated Schwartz formula shows estimated GFR (eGFR) by the serum creatinine with the enzymatic method in children [13]. The updated Schwartz formula overestimates the renal function in Japanese children and a new formula has been presented [14]. In the present study, eGFR was calculated as follows: eGFR (mL/min/1.73 m^2) = 110.2 × reference value of creatinine (mg/dL) / measured value of creatinine (mg/dL) + 2.93. Reference value of serum creatinine was showed by the following formulas by gender and *Ht* meant height (m). Creatinine of boys = $-1.259\,Ht^5 + 7.815\,Ht^4 - 18.57\,Ht^3 +$

$21.39\ Ht^2 - 11.71\ Ht + 2.628$, creatinine of girls = $-4.536\ Ht^5 + 27.16\ Ht^4 - 63.47\ Ht^3 + 72.43\ Ht^2 - 40.06\ Ht + 8.778$.

Statistical Analysis

The continuous variables were expressed as means ± standard deviation, and variables not following a normal distribution were expressed as median (minimum, maximum). Differences between boys and girls were compared by unpaired t-test or Mann-Whitney U test. Pearson's correlation test was performed to identify factors individually associated with CysC levels. Multivariable regression analysis was performed to examine variables associated with CysC, with adjustment for sex, height, BUN, SUA, HDL-C, LDL-C and TG, except for collinear variables.

To identify factors associated with both CysC and SUA, boys and girls were categorized into 4 groups according to the median levels of CysC (0.93 mg/L and 0.78 mg/L, respectively) and SUA (5.8 mg/dL and 4.5 mg/dL, respectively): group 1, low CysC and low SUA; group 2, high CysC and low SUA; group 3, low CysC and high SUA; and group 4, high CysC and high SUA. Mean serum lipid levels in the 4 groups were examined to evaluate the presence of serum lipid imbalance, which might suggest cardiometabolic risk. Data distributions of these subgroups were considered normal and differences between groups were analyzed using one-way ANOVA followed by Dunnet's test for post-hoc comparisons. All *p*-values were two-sided, and statistical significance was set at $p < 0.05$. Analyses were performed using IBM SPSS Statistics version 22.0 (IBM Co. SPSS Inc., Chicago, IL).

Results

Baseline Characteristics

Table 1 summarizes the characteristics of the study participants. There were significant differences between boys and girls in physical measurements, as expected, and laboratory data also showed significant gender differences, apart from serum levels of albumin, C-reactive protein, HDL-C and TG. The distributions of subjects by serum creatinine and CysC levels (Fig. 1) showed significant gender differences, which were more significant for CysC than for creatinine (Table 1). In contrast to CysC, the distribution of subjects with respect to creatinine had a wide range deviation.

Variables Associated with Serum CysC Levels

CysC significantly correlated with serum creatinine, and with male sex, as expected (Table 2). Importantly, CysC had a significant positive correlation with SUA among all subjects ($r = 0.546$, $p < 0.001$), as well as in boys and girls separately ($r = 0.333$ and 0.260, respectively, both $p < 0.001$, Fig. 2). There was a weak correlation between CysC and body height in all subjects ($r = 0.299$, $p < 0.001$), but no other factor showed significant correlation with CysC. In contrast, serum creatinine levels showed strong correlations with body height and weight ($r = 0.507$ and 0.431, respectively, both $p < 0.001$).

A multivariable regression analysis incorporating variables correlated with CysC (Table 3) found significant associations between CysC level and the following, after adjustment: male sex ($p < 0.001$), levels of SUA ($p < 0.001$) and TG ($p = 0.003$) among all subjects. The positive association of CysC and SUA was also significant in both boys and girls ($p < 0.001$ for each). The positive association of CysC with TG was observed separately in boys ($p = 0.017$), but not in girls ($p = 0.065$).

Clinical Significance of CysC and SUA

Boys and girls were categorized into 4 groups as described above. Fig. 2 shows the group categorization and table 4 summarizes characteristics of subjects in each subgroup. Boys in group 4 (CysC and SUA both high) showed significant differences in body height, weight, BMI and waist compared with those in group 1 (CysC and SUA both low). Girls in group 4 similarly showed significant differences between groups, except for body height ($p = 0.334$). Notably, boys in group 4 had the lowest HDL-C ($p = 0.017$) and the highest TG ($p = 0.008$), suggesting a possible subclinical abnormality of lipid metabolism that could represent cardiometabolic risk accumulation. We next compared TG/HDL-C ratio (Fig. 3), and found as expected that boys in group 4 had the highest TG/HDL-C ratio as compared with other subgroups ($p = 0.010$). Although differences between subgroups of girls were not statistically significant, girls in group 4 appeared to have lower HDL-C, higher TG and higher TG/HDL-C similar to those of boys in group 4.

Discussion

In this study, we examined the distribution of the serum CysC level of boys and girls in a cohort of 454 Japanese junior high school students, and demonstrated that the CysC level was significantly associated with the SUA level. Importantly, subjects with high levels of both CysC and SUA had the highest TG/HDL-C ratio. The association of CysC and subclinical lipid abnormalities suggests the presence of cardiometabolic risk accumulation in young adolescents, and the potential of CysC and SUA for surrogate markers to identify high-risk young subjects for future cardiovascular events.

A meta-analysis of diagnosis for renal function has demonstrated that CysC has higher sensitivity, likelihood ratio, and thus a higher diagnostic odds ratio than creatinine [15]. The distribution and deviation of CysC in our study also support the case for CysC as a new reference standard for renal function in young adolescents. On the contrary, the diagnostic accuracy of serum creatinine is affected by inter-individual variations in muscle mass as well as the tubular secretion of creatinine [15]. Our study also showed strong correlations between serum creatinine and the physical attributes of body height, weight and waist, compared with those of CysC.

There have been causal associations between hyperuricemia and metabolic syndrome [16], insulin resistance, and the development of systemic hypertension [17]. Moreover, gout [18] and elevated SUA [19] have been significantly associated with cardiovascular events, and also correlated with cerebral microbleeds in patients with ischemic stroke [20]. There has been a critical controversy, however, whether SUA is an independent risk of cardiovascular disease or, on the contrary, SUA can protect against cardiovascular disease by its antioxidant property [21]. The antioxidant property of SUA has been beneficial for neurological outcomes in patients with acute ischemic stroke [22, 23]. Furthermore, a recent study has demonstrated that SUA is not an independent predictor of cardiovascular mortality [24]. Therefore, we need to identify high-risk individuals among young subjects with hyperuricemia. The present study provides the new insight that hyperuricemia is associated with subclinical lipid abnormalities in young subjects with high CysC. The concomitant elevation of CysC and SUA, rather than the presence of hyperuricemia alone, could potentially identify subjects at high risk for the future development of cardiometabolic diseases among junior high school students.

Gender differences in ischemic heart disease and cardiometabolic risks have been demonstrated [25, 26]. SUA levels have been found to be higher in men than in women, and elevated SUA is an independent risk factor for carotid artery plaque only in men [12]. Estrogen is known to protect against atherosclerosis in women. The gender differences of TG/HDL-C ratio in subgroups (Fig. 3) can be explained, at least in part, by the estrogen effects, which are beneficial not only on lipid metabolism [27], but also on the renal excretion of uric acid [28, 29].

TG/HDL-C ratio has been correlated with small dense LDL (sdLDL) particles in adult patients with type 2 diabetes mellitus [30], and associated with cardiovascular events and all-cause mortality in adults [31]. Moreover, TG/HDL-C ratio was useful in detecting metabolic syndrome in obese children [32]. Interestingly, adults with high TG/HDL-C ratio showed increased prevalence of chronic kidney disease [33, 34], suggesting a possible association between dyslipidemia and renal dysfunction as similarly shown in the present study. In light of these findings, this study may highlight the importance of the dual evaluation of CysC and SUA for identifying high-risk subjects who should receive early attention to primary prevention of cardiometabolic diseases.

Limitations

First, this was a single-prefecture survey of health screening in a cohort of Japanese junior high school students and was limited by the small number of subjects. Second, lifestyles, family histories and medical histories, including birth records or development of subjects, were not available in this study. Third, the blood examination was performed only at a single time point; thus, we had no follow-up data on the outcomes of enrolled subjects. Long-term longitudinal studies are, therefore, warranted to determine the role of CysC and SUA in young adolescents for predicting the development of cardiovascular diseases.

Conclusions

Serum CysC level was significantly associated with SUA in a cohort of Japanese junior high school students. The concomitant elevation of serum CysC and SUA levels may identify subjects with subclinical lipid metabolism dysregulation. This could be important for primary prevention against the subsequent development of cardiovascular disease.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Figure legends

Fig. 1. Histogram of number of boys and girls per serum levels of creatinine and cystatin C (CysC), and estimated glomerular filtration rate (eGFR). CysC of boys and girls showed normal distributions with narrower deviations as compared with those of creatinine. CysC level of boys was significantly higher as compared with that of girls.

Fig. 2. Correlation between serum cystatin C and uric acid with 95% confidence interval of the regression line. Dotted lines represent median values of each examination.

Fig. 3. The ratios of TG to HDL-C in subgroups of boys and girls. A subgroup of boys with high cystatin C and high uric acid had the highest TG/HDL-C ratio. Girls also had similar tendency as boys but statistically no difference between subgroups. TG, triglyceride; HDL-C, high-density lipoprotein cholesterol.

Table 1. Characteristics of study participants

		All (n = 454)	Boys (n = 216)	Girls (n = 238)	p
Height	(cm)	158.6 ± 7.4	161.3 ± 8.1	156.1 ± 5.6**	<0.001
Weight	(kg)	49.0 ± 8.8	49.9 ± 8.8	48.1 ± 8.8*	0.034
Waist	(cm)	66.3 ± 7.6	65.7 ± 6.3	66.8 ± 8.7	0.026
Waist/Height		0.42 ± 0.05	0.41 ± 0.04	0.43 ± 0.05**	0.127
BMI	(kg/m^2)	19.4 ± 3.0	19.1 ± 2.6	19.7 ± 3.1	<0.001
SBP	(mmHg)	107.9 ± 10.8	109.1 ± 11.0	106.9 ± 10.5*	0.037
DBP	(mmHg)	60.6 ± 7.7	60.5 ± 7.7	60.7 ± 7.6	0.778
Total protein	(g/dL)	7.6 ± 0.4	7.5 ± 0.4	7.6 ± 0.4**	<0.001
Albumin	(g/dL)	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.3	0.699
BUN	(mg/dL)	12.8 ± 2.8	13.6 ± 2.9	12.1 ± 2.6**	<0.001
Creatinine	(mg/dL)	0.65 ± 0.13	0.69 ± 0.14	0.60 ± 0.09**	<0.001
eGFR	(mL/min/ 1.73 m^2)	95.1 ± 15.0	94.8 ± 13.7	95.3 ± 16.3	0.737
Uric acid	(mg/dL)	5.0 ± 1.1	5.7 ± 1.0	4.4 ± 0.8**	<0.001
CRP	(mg/dL)	0.01 (0.00, 1.14)	0.02 (0.00, 0.72)	0.01 (0.00, 1.14)	0.014
HDL-C	(mg/dL)	67.1 ± 13.9	66.5 ± 14.5	67.6 ± 13.3	0.393
LDL-C	(mg/dL)	87.5 ± 23.7	82.4 ± 23.0	92.1 ± 23.6**	<0.001
Triglyceride	(mg/dL)	64.5 (20.0, 352.0)	62.5 (20.0, 302.0)	67.0 (22.0, 352.0)	0.055
Cystatin C	(mg/L)	0.84 ± 0.12	0.92 ± 0.10	0.77 ± 0.08**	<0.001

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; BUN, blood urea nitrogen; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Values are given as mean ± standard deviation or median (minimum, maximum). *$p < 0.05$, **$p < 0.001$ vs. boys.

Table 2. Association between variables and serum cystatin C levels

	All		Boys		Girls	
	r	*p*	*r*	*p*	*r*	*p*
Sex	0.645	<0.001				
Height	0.299	<0.001	0.124	0.069	0.063	0.331
Weight	0.129	0.006	0.101	0.141	0.070	0.284
Waist	-0.009	0.851	0.044	0.520	0.057	0.378
Waist/Height	-0.135	0.004	-0.034	0.621	0.037	0.567
BMI	-0.041	0.384	0.031	0.649	0.040	0.536
SBP	0.078	0.099	0.051	0.460	-0.021	0.751
DBP	-0.023	0.630	-0.058	0.398	0.029	0.660
BUN	0.211	<0.001	0.174	0.011	-0.099	0.128
Creatinine	0.473	<0.001	0.372	<0.001	0.301	<0.001
Uric acid	0.546	<0.001	0.333	<0.001	0.260	<0.001
CRP	0.05	0.289	0.027	0.698	0.012	0.858
HDL-C	-0.081	0.085	-0.101	0.140	-0.035	0.587
LDL-C	-0.145	0.002	-0.002	0.980	-0.036	0.576
Triglyceride	0.083	0.078	0.201	0.003	0.150	0.021

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; BUN, blood urea nitrogen; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *r*, correlation coefficient.

Table 3. Variables associated with serum cystatin C by multivariate regression

	B (95%CI)	β	p
All			
Sex	0.115 (0.095 to 0.134)	0.491	<0.001
Height	0.000 (-0.001 to 0.001)	0.016	0.684
BUN	0.002 (-0.001 to 0.001)	0.053	0.145
Uric acid	0.026 (0.017 to 0.035)	0.248	<0.001
HDL-C	0.000 (-0.001 to 0.001)	0.006	0.877
LDL-C	0.000 (0.000 to 0.000)	-0.036	0.312
Triglyceride	0.000 (0.000 to 0.001)	0.111	0.003
Boys			
Height	0.000 (-0.002 to 0.002)	0.012	0.867
BUN	0.006 (0.001 to 0.011)	0.167	0.012
Uric acid	0.027 (0.014 to 0.041)	0.280	<0.001
HDL-C	0.000 (-0.001 to 0.001)	-0.022	0.739
LDL-C	0.000 (-0.001 to 0.000)	-0.036	0.584
Triglyceride	0.000 (0.000 to 0.001)	0.162	0.017
Girls			
Height	0.001 (-0.001 to 0.002)	0.046	0.480
BUN	-0.002 (-0.006 to 0.001)	-0.081	0.213
Uric acid	0.022 (0.010 to 0.034)	0.240	<0.001
HDL-C	0.000 (0.000 to 0.001)	0.050	0.457

LDL-C	0.000 (-0.001 to 0.000)	-0.067	0.297
Triglyceride	0.000 (0.000 to 0.001)	0.129	0.065

Adjusted for sex, height, BUN, HDL-C, LDL-C and triglycerides.

Abbreviations: BUN, blood urea nitrogen; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; B, partial regression coefficient; β, standardized partial regression coefficient; CI, confidence interval.

Table 4. Characteristics of subjects categorized by serum levels of cystatin C and uric acid

a. Boys

	Group 1	Group 2	Group 3	Group 4	*p*
CysC	< 0.93	≥ 0.93	< 0.93	≥ 0.93	
SUA	< 5.8	< 5.8	≥ 5.8	≥ 5.8	
n	74	41	45	56	
Height	159.6 ± 9.0*	159.0 ± 8.1*	163.4 ± 6.6	163.6 ± 7.2	0.003
Weight	47.1 ± 7.9*	47.3 ± 8.1*	52.8 ± 7.3	53.2 ± 9.8	<0.001
BMI	18.4 ± 2.1*	18.6 ± 2.3	19.7 ± 2.3	19.8 ± 3.3	<0.001
Waist	64.2 ± 5.0*	64.7 ± 6.4	67.5 ± 5.7	67.1 ± 7.5	0.007
Creatinine	0.64 ± 0.13*	0.67 ± 0.12*	0.68 ± 0.12*	0.78 ± 0.12	<0.001
HDL-C	70.6 ± 16.4*	65.7 ± 11.6	64.2 ± 13.9	63.5 ± 13.2	0.024
LDL-C	82.8 ± 22.2	85.8 ± 18.9	80.0 ± 18.2	81.4 ± 29.5	0.671
Triglyceride	60.5 ± 26.5*	78.6 ± 52.4	70.8 ± 32.8	80.1 ± 38.3	0.012

b. Girls

	Group 1	Group 2	Group 3	Group 4	*p*
CysC	< 0.78	≥ 0.78	< 0.78	≥ 0.78	
SUA	< 4.5	< 4.5	≥ 4.5	≥ 4.5	
n	76	34	55	73	
Height	155.5 ± 4.9*	156.1 ± 6.7*	155.8 ± 5.1	157.1 ± 6.1	0.334
Weight	46.5 ± 6.2*	44.9 ± 8.1*	49.6 ± 7.0	50.3 ± 11.6	0.004

BMI	19.2±2.4	18.3 ± 2.3*	20.4 ± 2.6	20.3 ± 4.3	0.002
Waist	65.3 ± 6.3	64.0 ± 6.5*	68.3 ± 6.8	68.6 ± 11.8	0.012
Creatinine	0.58 ± 0.08*	0.60 ± 0.10	0.60 ± 0.09	0.63 ± 0.10	0.002
HDL-C	69.7 ± 14.1	67.5 ± 12.9	67.1 ± 13.1	65.9 ± 12.9	0.380
LDL-C	92.9 ± 25.5	87.2 ± 21.3	93.9 ± 23.4	92.2 ± 22.7	0.598
Triglyceride	70.7 ± 34.8	79.8 ± 38.1	74.3 ± 38.7	85.2 ± 51.1	0.181

Abbreviations: CysC, cystatin C; SUA, serum uric acid; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Values are given as mean ± standard deviation. *$p < 0.05$ vs. group 4.

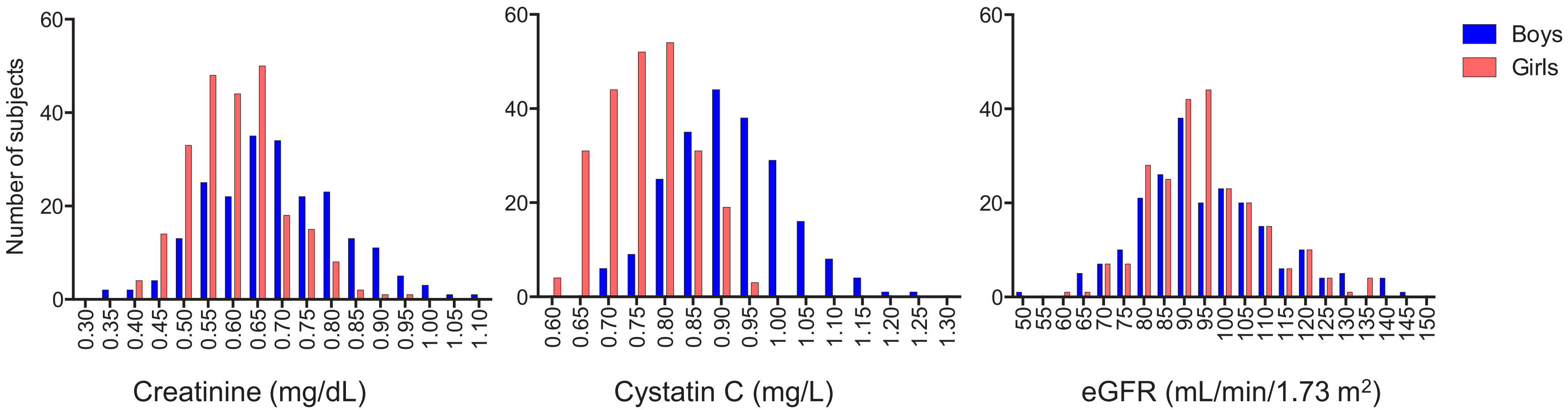
Number of subjects
60
40
20
0
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
1.05
1.10
Creatinine (mg/dL)
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
1.05
1.10
1.15
1.20
1.25
1.30
Cystatin C (mg/L)
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
eGFR (mL/min/1.73 m2)
Boys
Girls

Figure 1

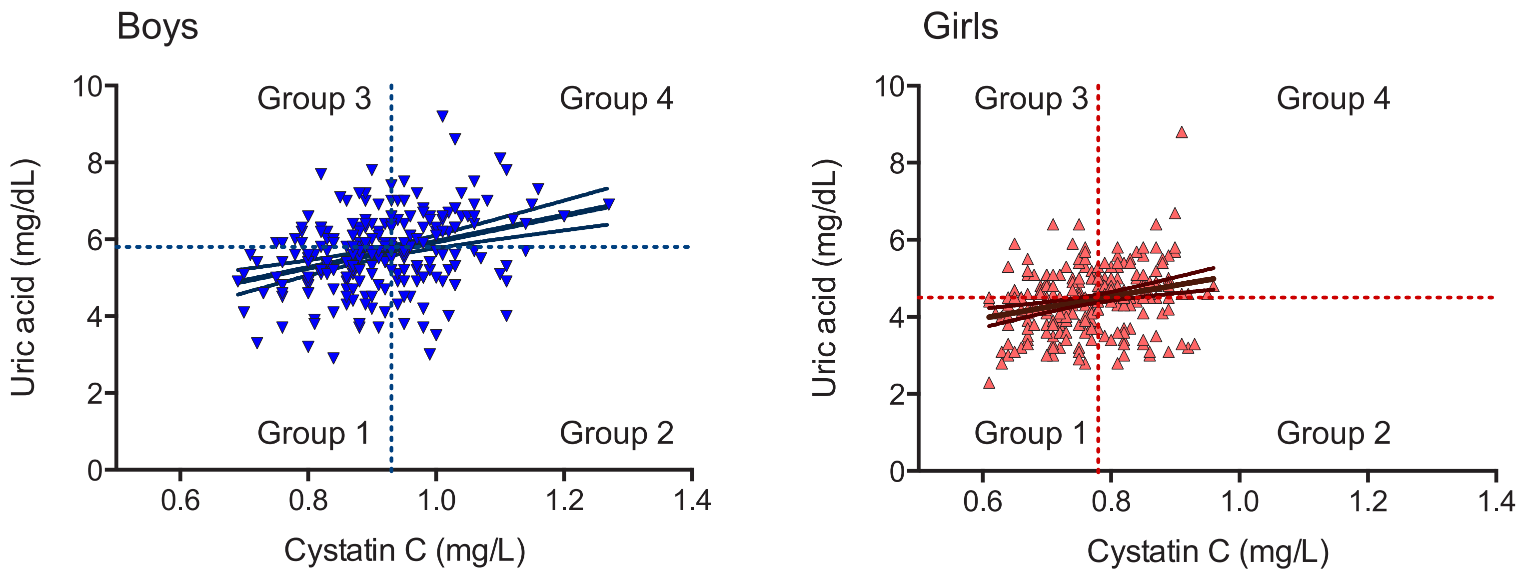
Boys
Uric acid (mg/dL)
10
8
6
4
2
0
Group 3
Group 4
Group 1
Group 2
0.6
0.8
1.0
1.2
1.4
Cystatin C (mg/L)
Girls
Uric acid (mg/dL)
10
8
6
4
2
0
Group 3
Group 4
Group 1
Group 2
0.6
0.8
1.0
1.2
1.4
Cystatin C (mg/L)

Figure 2

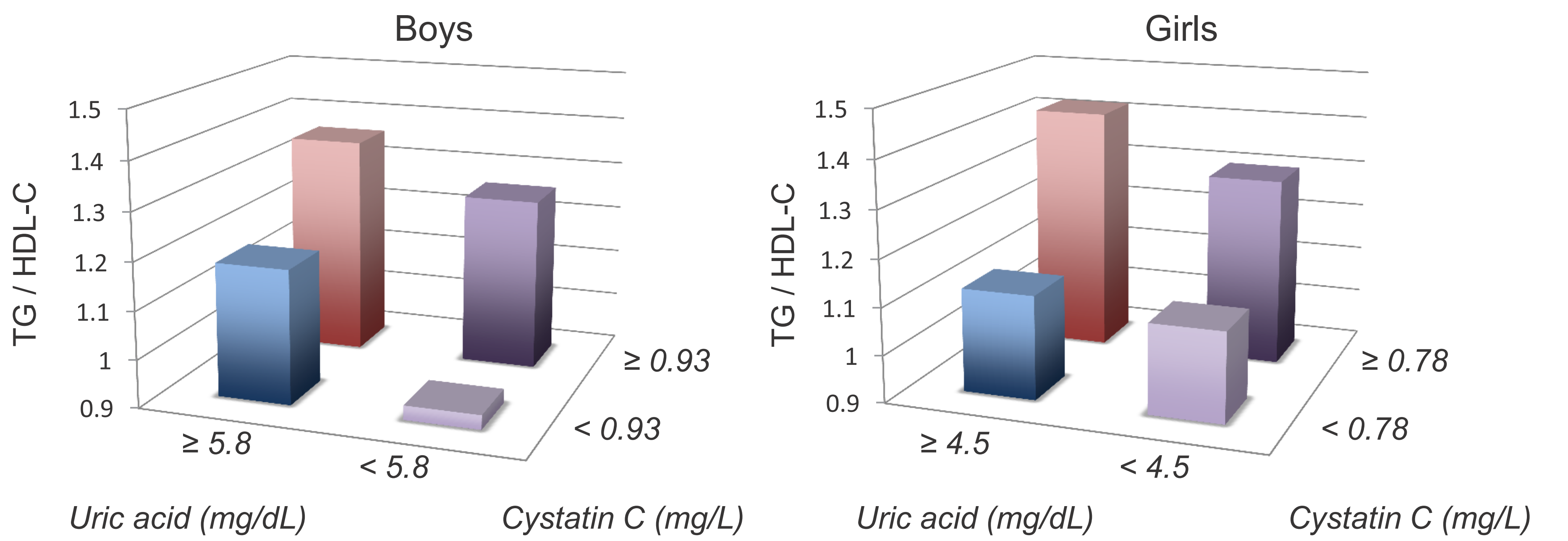
Boys
TG / HDL-C
1.5
1.4
1.3
1.2
1.1
1
0.9
≥ 5.8
< 5.8
≥ 0.93
< 0.93
Uric acid (mg/dL)
Cystatin C (mg/L)
Girls
TG / HDL-C
1.5
1.4
1.3
1.2
1.1
1
0.9
≥ 4.5
< 4.5
≥ 0.78
< 0.78
Uric acid (mg/dL)
Cystatin C (mg/L)

Figure 3