Urinary Cystatin C as a Potential Risk Marker for Cardiovascular Disease and Chronic Kidney Disease in Patients with Obesity and Metabolic Syndrome

Noriko Satoh-Asahara,*† Takayoshi Suganami,‡ Takafumi Majima,* Kazuhiko Kotani,*§ Yasuhisa Kato,‖ Rika Araki,¶ Kazunori Koyama,**†† Taiichiro Okajima,‡‡ Makito Tanabe,‡‡ Mariko Oishi,§§ Akihiro Himeno,*† Shigeo Kono,† Akira Sugawara,‖‖ Masakazu Hattori,* Yoshihiro Ogawa,‡ Akira Shimatsu,* and the Japan Obesity and Metabolic Syndrome Study (JOMS) Group

Summary

Background and Objectives Obesity and metabolic syndrome (MS) increase the risk of cardiovascular disease (CVD), chronic kidney disease (CKD), and all-cause mortality. Serum cystatin C (S-CysC), a marker of GFR, has been shown to be associated with CVD and CKD. This study was designed to elucidate the association of urinary CysC (U-CysC), a marker of renal tubular dysfunction, with CVD and CKD risk factors in patients with obesity and MS.

Design, setting, participants, & measurements The U-CysC-creatinine ratio (UCCR) was examined in 343 Japanese obese outpatients enrolled in the multi-centered Japan Obesity and Metabolic Syndrome Study.

Results UCCR was positively correlated with urine albumin-creatinine ratio (UACR) and S-CysC and negatively correlated with estimated GFR (eGFR). Among obese patients, UCCR was significantly higher in MS patients than in non-MS patients. UCCR had significant correlations with the number of components of MS and arterial stiffness, all of which are CVD predictors, similarly to UACR ($P < 0.05$). Interestingly, diet- and exercise-induced weight reduction for 3 months significantly decreased only UCCR among all of the renal markers examined ($P < 0.01$), in parallel with the decrease in BMI, HbA1c, and arterial stiffness, suggesting the beneficial effect of weight reduction on renal tubular dysfunction.

Conclusions This study demonstrates that UCCR is significantly associated with renal dysfunction, the severity of MS, arterial stiffness, and weight change in obese patients. The data of this study suggest that U-CysC could serve as a CVD and CKD risk factor in patients with obesity and MS.
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*Division of Diabetic Research, Clinical Research Institute, †Diabetes Center, and ‖Department of Nephrology, National Hospital Organization, Kyoto Medical Center, Kyoto, Japan; ‡Department of Molecular Medicine and Metabolism, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; §Department of Clinical Laboratory Medicine, Jichi Medical University, Shimotsuke, Japan; ‖Diabetes Center, National Hospital Organization, Nagoya Medical Center, Nagoya, Japan; ¶Internal Medicine, National Hospital Organization, Mie Hospital, Tsu, Japan; **Internal Medicine, National Hospital Organization, Tokyo Medical Center, Tokyo, Japan; ††University of Health and Welfare, Mita Hospital, Tokyo, Japan; ‡‡Department of Internal Medicine and Department of Clinical Research, National Hospital Organization, Kokura Medical Center, Kitakyu-syu, Japan; and §§Oishi Clinic, Kyoto, Japan

Correspondence: Dr. Noriko Satoh-Asahara, Division of Diabetic Research, Clinical Research Institute, National Hospital Organization, Kyoto Medical Center, 1-1 Fukakusa Mukaihata-cho, Fushimi-ku, Kyoto 612-8555, Japan. Phone: 81-75-641-9161; Fax: 81-75-645-2781; E-mail: norikos@oregano. ocn.ne.jp

Introduction

Obesity and metabolic syndrome (MS), a cluster of multiple risk factors for atherosclerosis such as obesity, elevated BP, elevated glucose, and atherogenic dyslipidemia, increase the risk of all-cause mortality and cardiovascular morbidity and mortality (1,2). Chronic kidney disease (CKD), which is defined as renal damage or GFR < 60 ml/min per 1.73 m² for at least 3 months (3), is also known to be an independent risk for cardiovascular diseases (CVD) (4,5). Recent epidemiologic studies revealed a close association of MS and obesity with CKD (6–8). It is speculated that CVD and CKD share common pathophysiologic bases involving metabolic abnormalities, endothelial dysfunction, oxidative stress, and chronic inflammation (9). However, conventional CVD risk factors underestimate the risk of CVD in patients with CKD, implying novel mechanisms linking CVD and CKD (10). It is, therefore, important to identify new biomarkers to evaluate the progression of CVD and CKD in patients with obesity and MS.

Microalbuminuria is an established biomarker that reflects the decline in GFR and a predictive and independent biomarker for all-cause mortality and CVD events (11,12). Cystatin C (CysC), a 13-kD endogenous cysteine proteinase inhibitor, is ubiquitously expressed, filtrated freely by the glomeruli, and reabsorbed by the proximal tubules (13,14). Serum CysC (S-CysC) is also a sensitive marker for detecting reduced GFR and is a stronger predictor of the risk of death and cardiovascular events in elderly persons than serum creatinine (14–16). On the other hand, the level of urinary CysC level (U-CysC) has been recognized as a marker of renal tubular dysfunction (17–19). Recently, several studies have provided evidence for the involvement of renal tubular dysfunction as well as glomerulopathy in the CKD progression in diabetic patients with macrovascular diseases (20,21). However, it is unknown whether renal tubular dysfunction is associated with the development of renal damage and CKD in obesity and MS. Moreover, whether U-CysC is associated with the conventional

risk factors of CVD and CKD in patients with obesity and MS has never been addressed.

The Japan Obesity and Metabolic Syndrome Study (JOMS) is a prospective and cross-sectional multicenter study that involves several National Hospital Organization hospitals in Japan. In a series of previous studies, we demonstrated that as a new atherogenic index, cardio-ankle vascular index (CAVI) and serum amyloid A-oxidized LDL are useful for the evaluation and management of the CVD risks in patients with obesity and MS (22,23). We also reported that the markers are sensitive for detecting improvement of CVD risks during weight reduction. Thus, JOMS would provide unique opportunities to investigate the pathophysiology of obesity-related renal dysfunction by cross-sectional and prospective designs.

In this study, we examined the relationships of U-CysC with CVD and CKD risk factors and the significance of renal tubular dysfunction in the progression of CVD and CKD in obese patients. Among the obese patients, we compared the U-CysC levels by classifying the patients as either having MS (MS group) or not (non-MS group) or by stratifying according to the number of MS traits. Because weight reduction improves glomerular hemodynamics and reduces urine albumin excretion (24–26), we also studied the effect of weight-reduction therapy on U-CysC. Here, we show a significant correlation of U-CysC with CVD and CKD risk factors in obese patients. Interestingly, diet- and exercise-induced weight reduction effectively suppressed the increased U-CysC in obese patients. The data of this study suggest that U-CysC may serve as a potential biomarker that reflects the progression of CVD and CKD in patients with obesity and MS.

Materials and Methods
Subjects

A total of 343 Japanese obese outpatients (152 men and 191 women; mean age, 52.1 years) were consecutively enrolled in a multi-center study (JOMS), which involved five National Hospital Organization hospitals (Kyoto, Tokyo, Nagoya, and Kokura Medical Centers and Mie Hospital) and the Oishi Clinic in Japan as part of a study conducted by the Policy Based Medical Service Network for Endocrine and Metabolic Diseases during the period from October 2005 to March 2007 (22,23). Each institution's ethical committee approved this study, and all of the patients gave their written informed consent. The JOMS study has been registered in the University Hospital Medical Information Network system (study identification UMIN000000559), which is open to the public. It was designed to assess the characteristics of MS and the effectiveness of weight-reduction therapy at preventing CVD in Japanese obese subjects.

We recruited obese subjects with a body mass index (BMI) of ≥ 25 kg/m^2. The exclusion criteria were no previous history of CVD, other vascular diseases, apparent renal disease, or severe liver dysfunction. The patients were classified as having MS (MS group) or not (non-MS group) according to the criteria proposed by the United States National Cholesterol Education Program-Adult Treatment Panel III with a slight modification on waist circumference, as a representative criteria of this syndrome (27). The criteria for waist circumference was set at ≥ 85 cm for men and ≥ 90 cm for women, on the basis of the criteria of the National Metabolic Syndrome Criteria Study Group of Japan (28).

Data Collection and Laboratory Measurements

At the beginning of and 3 months after weight-reduction therapy, we measured MS-related parameters (BMI, waist circumference, systolic [SBP], and diastolic BP [DBP]); blood parameters; and CAVI, an index of arterial stiffness (22). Fasting blood samples were drawn in the absence of prescription drugs, and fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), immunoreactive insulin (IRI), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides (TG) were determined according to the standard procedures (22). Serum levels of C-reactive protein (CRP), an inflammatory marker, and leptin and adiponectin, representative adipocytokines, were also determined (22). Urinary albumin (U-Alb) and urinary β-2 microglobulin (U-B2MG) were assessed quantitatively by the latex particle enhanced immunoturbidimetric method (Eiken, Japan). S-CysC was determined by a latex particle enhanced immunoturbidimetric assay (Ikagaku, Kyoto, Japan) (17). U-CysC was measured with an ELISA as described (17). The urine albumin-creatinine ratio (UACR) and urine U-CysC-creatinine ratio (UCCR) were calculated (12,17). Urinary N-acetyl-β-D-glucosaminidase (U-NAG) was measured using a colorimetric assay (Shionogi, Japan). Estimated GFR (eGFR) was calculated with the new Japanese coefficient for the abbreviated Modification of Diet in Renal Disease Study equation, including a correction factor of 0.739 for women (29). According to the United States National Kidney Foundation guidelines, CKD was defined as an eGFR <60 ml/min per 1.73 m^2 and was classified into five stages according to e-GFR levels (3). CAVI, a newly developed indicator of arterial stiffness that is independent of BP, was determined using a Vasera VS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan), as described previously (22).

Weight-Reduction Therapy

Of those enrolled in this study, 182 obese patients (81 men and 101 women; mean age, 52.5 years; mean BMI, 31.1) undertook weight-reduction therapy through lifestyle modifications to reduce energy intake and increase physical activity for 3 months as described previously (22,23). We defined those who were able to reduce their body weight by 5% or more as successful in weight reduction and those who reduced their body weight by less than 5% as unsuccessful (22).

Statistical Analyses

The data are presented as the means ± SEM, and $P < 0.05$ was considered statistically significant. The t test and the χ^2 test were used as appropriate for assessing intergroup differences. Analysis of covariance (ANCOVA) adjusted for gender and age was performed to assess differences in the mean levels of renal parameters stratified according to the number of MS components and the five categories of eGFR levels. A two-way repeated measured ANOVA was performed to compare changes in parameters over 3 months between patients with successful and unsuccessful in weight reduction. Pearson's correlation coefficient test was used to observe the correlations of renal parameters with metabolic parameters in all patients and of the changes in renal parameters with those in metabolic parameters during weight reduction. A stepwise multivariate regression analysis was performed to explore the factors related to the baseline levels and the changes in UCCR in all of the patients. All of the statistical analyses were performed using StatView version 5.0 for Windows (SAS Institute Inc.).

Results

Baseline Clinical Characteristics

Table 1 summarizes the characteristics of the study cohort and compares the patients with and without MS. The prevalence of MS among the obese patients was 73.2%. As expected, age, waist circumference, SBP, DBP, FPG, HbA1c, IRI, and TG were significantly higher and HDL-C level was significantly lower in the MS group than in the non-MS group ($P < 0.05$). In this study, BMI, LDL-C, leptin,

Table 1. Baseline characteristics of the study obese patients stratified by the presence of MS diagnosed according to the National Cholesterol Education Program-Adult Treatment Panel III criteria

	Total	Non-MS	MS
Male/female	152/191	33/59	119/132
Age (years)	52.1 ± 0.8	48.8 ± 1.5	53.2 ± 0.9[a]
BMI (kg/m^2)	31.2 ± 0.3	30.7 ± 0.7	31.4 ± 0.4
Waist circumference (cm)	100 ± 0.8	97.6 ± 1.9	101 ± 0.8[a]
Systolic blood pressure (mmHg)	139 ± 0.9	130 ± 1.5	143 ± 1.1[b]
Diastolic blood pressure (mmHg)	82.5 ± 0.7	77.6 ± 1.2	84.3 ± 0.8[b]
Fasting plasma glucose (mg/dl)	132 ± 2.7	108 ± 3.8	140 ± 3.3[b]
HbA1c (%)	6.72 ± 0.1	5.99 ± 0.1	6.98 ± 0.1[b]
IRI (μU/ml)	21.0 ± 1.7	15.4 ± 1.9	23.0 ± 2.1[a]
Triglyceride (mg/dl)	173 ± 7.1	126 ± 5.6	190 ± 9.2[b]
HDL-C (mg/dl)	53.4 ± 0.7	58.6 ± 1.3	51.5 ± 0.8[b]
LDL-C (mg/dl)	124 ± 1.7	125 ± 2.9	124 ± 2.1
Leptin (ng/ml)	14.3 ± 0.7	13.4 ± 1.1	14.6 ± 0.8
Adiponectin (μg/ml)	6.48 ± 0.2	6.64 ± 0.3	6.43 ± 0.2
CRP (μg/ml)	1.41 ± 0.1	1.23 ± 0.1	1.47 ± 0.1
eGFR	82.6 ± 1.4	85.4 ± 2.2	81.7 ± 1.7
S-CysC (mg/L)	0.84 ± 0.02	0.78 ± 0.03	0.86 ± 0.02[a]
UACR (mg/g·Cr)	99.2 ± 23	37.5 ± 9.7	122 ± 31[a]
UCCR	0.77 ± 0.05	0.61 ± 0.02	0.82 ± 0.07[a]
U-B2MG (μg/L)	186 ± 27	117 ± 20	212 ± 36[a]
U-NAG (U/L)	4.17 ± 0.3	2.91 ± 0.3	4.63 ± 0.4[a]
CAVI	7.85 ± 0.1	7.41 ± 0.2	8.02 ± 0.1[b]
Proportion of			
hypertension (%)	77.0	46.7	93.2[b]
diabetes (%)	60.6	21.7	80.1[b]
dyslipidemia (%)	62.2	23.9	81.3[b]
chronic kidney disease (%)	15.7	5.4	19.5[b]
taking calcium antagonist (%)	25.4	10.9	32.7[b]
taking ACE/ARB (%)	31.2	13.1	40.2[b]
taking antidiabetic medications (%)	45.1	19.6	57.8[b]
taking statins (%)	25.0	6.5	34.3[b]

The data are expressed as the means ± SEM. ACE, angiotensin-converting enzyme; ARB, angiotensin II type 1 receptor blocker.
[a]$P < 0.05$ *versus* non-MS.
[b]$P < 0.001$ *versus* non-MS.

and adiponectin levels did not differ significantly between the two groups. Moreover, CAVI was significantly higher in the MS group than in the non-MS group ($P < 0.001$), which is consistent with our previous report (22). Although eGFR did not differ significantly between the MS and non-MS groups, UCCR was significantly higher in the MS group than in the non-MS group ($P < 0.05$). S-CysC, UACR, U-B2MG, and U-NAG were also significantly higher in the MS group than the non-MS group ($P < 0.05$) (Table 1). In this study, the prevalence of hypertension, diabetes, dyslipidemia, and CKD was higher in the MS group than the non-MS group (proportion of CKD: non-MS group, 5.4%; MS group, 19.5%).

Correlation between Renal Markers and Metabolic Parameters

Among the renal markers, logUCCR was significantly correlated with logUACR ($P < 0.01$). Interestingly, both logUACR and logUCCR had significant positive correlations with age, SBP, HbA1c, adiponectin, CAVI, and other renal markers (S-CysC, logU-B2MG, and logU-NAG) and had a negative correlation with eGFR ($P < 0.05$) (Table 2). Only logUCCR, but not logUACR, was significantly correlated with leptin ($P < 0.05$). On the other hand, only logUACR, but not logUCCR was significantly correlated with

BMI, FPG, and IRI ($P < 0.05$). In this study, eGFR was negatively correlated with age, adiponectin, CAVI, and renal markers (S-CysC, logUACR, logUCCR, and logU-B2MG) and was positively correlated with BMI, waist circumference, and DBP ($P < 0.05$). S-CysC was positively correlated with age, waist circumference, leptin, and adiponectin and was negatively correlated with HDL-C and eGFR ($P < 0.05$). logU-B2MG was positively correlated with age, SBP, DBP, HbA1c, leptin, and adiponectin and negatively correlated with LDL-C. logU-NAG was positively correlated with BMI, waist circumference, FPG, HbA1c, leptin, and CRP and negatively correlated with HDL-C and adiponectin ($P < 0.05$). CAVI was positively correlated with S-CysC, logUACR, logUCCR, and logU-B2MG and negatively correlated with eGFR ($P < 0.01$) (Table 2). Because CAVI demonstrated a strong significant correlation with age, as previously reported (22), age-adjusted CAVI was analyzed with renal markers (data not shown). Importantly, only logUACR and logUCCR were significantly correlated with age-adjusted CAVI (logUACR, $r = 0.205$; logUCCR, $r = 0.185$, $P < 0.001$), whereas eGFR, S-CysC, logU-B2MG, and logU-NAG were not correlated with age-adjusted CAVI.

After adjusting for gender, age, BMI, SBP, FPG, IRI, TG, HDL-C, and LDL-C, which were selected as gen-

Table 2. Baseline correlations of eGFR, S-CysC, logUACR, logUCCR, logU-B2MG, and logU-NAG with metabolic parameters

	eGFR	S-CysC	logUACR	logUCCR	logU-B2MG	logU-NAG
				r		
Age	−0.458[a]	0.377[a]	0.321[a]	0.221[a]	0.156[b]	−0.001
BMI	0.272[a]	0.011	−0.143[b]	0.003	0.032	0.133[c]
Waist circumference	0.123[c]	0.111[c]	−0.102	0.028	0.038	0.131[c]
Systolic blood pressure	0.008	0.086	0.198[a]	0.115[c]	0.147[b]	0.062
Diastolic blood pressure	0.112[c]	−0.083	0.050	−0.003	0.120[c]	0.015
Fasting plasma glucose	−0.025	0.018	0.257[a]	0.083	0.058	0.214[a]
HbA1c	0.065	0.049	0.245[a]	0.167[b]	0.138[c]	0.348[a]
IRI	0.007	−0.010	0.140[c]	0.098	0.046	0.085
Triglyceride	−0.083	0.080	0.043	0.001	−0.012	−0.061
HDL-C	0.045	−0.144[b]	0.020	0.006	−0.045	−0.155[b]
LDL-C	0.058	−0.022	0.001	−0.009	−0.109[c]	−0.009
Leptin	−0.029	0.282[a]	0.106	0.282[a]	0.203[a]	0.108[c]
Adiponectin	−0.136[c]	0.147[b]	0.218[a]	0.201[a]	0.146[b]	−0.109[c]
CRP	0.019	0.048	0.006	−0.026	0.035	0.126[c]
eGFR	–	−0.573[a]	−0.208[a]	−0.151[b]	−0.119[c]	0.062
S-CysC	−0.573[a]	–	0.312[a]	0.376[a]	0.292[a]	0.109[c]
logUACR	−0.208[a]	0.312[a]	–	0.233[a]	0.321[a]	0.180[b]
logUCCR	−0.151[b]	0.376[a]	0.233[a]	–	0.341[a]	0.180[b]
CAVI	−0.419[a]	0.328[a]	0.373[a]	0.200[a]	0.178[b]	0.031

The data were analyzed by Pearson's correlation coefficient test.
[a]$P < 0.001$.
[b]$P < 0.01$.
[c]$P < 0.05$.

eral CVD risk factors to avoid using variables that demonstrate high colinearity with each other, only logUCCR and logUACR had significant associations with age-adjusted CAVI (logUACR, β = 0.203; logU-CCR, β = 0.173, $P < 0.01$) (Supplemental Table 1). S-CysC, logU-B2MG, and logU-NAG had no such associations with CAVI (Supplemental Table 1). A stepwise multivariate regression analysis revealed that age, HbA1c, and leptin are independently associated with logUCCR ($P < 0.001$). On the other hand, age, SBP, and HbA1c were independently associated with logUACR ($P < 0.01$) (Supplemental Table 2).

Renal Markers Stratified According to the Prognostic Stages of CKD and the Number of MS Traits

ANCOVA revealed that the mean levels of UCCR were increased in accordance with the prognostic stage of CKD in obese patients ($P < 0.001$) (Figure 1). There was also a significant increase in S-CysC and UACR in accordance with the prognostic stage of CKD ($P < 0.001$). We also examined the mean levels of eGFR, S-CysC, UACR, and UCCR, which were stratified according to the number of MS risk factors in all patients. When both gender and age were taken into account, UCCR was significantly associated with the number of MS risk factors as well as UACR ($P < 0.05$), whereas eGFR and S-CysC were not (Figure 2).
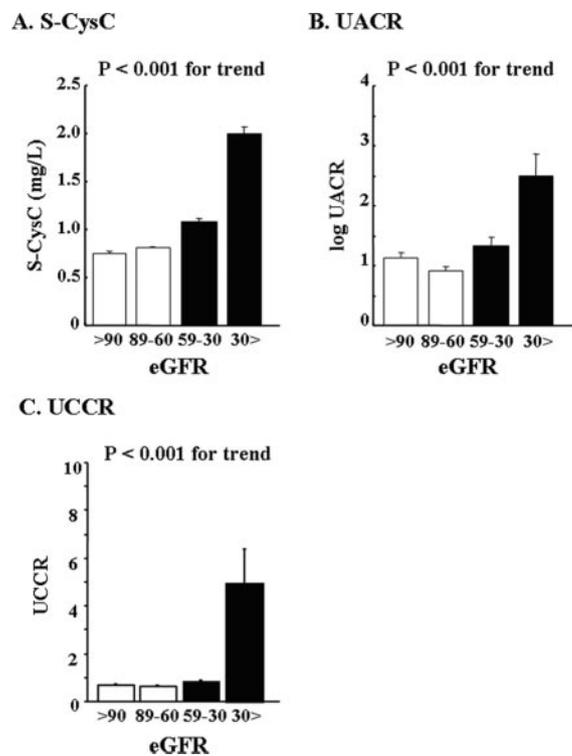

Figure 1. | **The values of S-CysC (A), UACR (B), and UCCR (C) stratified according to the prognostic stages of CKD after gender and age adjustment.** The data are shown as the means ± SEM. The differences between groups were assessed by ANCOVA. The open and closed bars indicate CKD and non-CKD according to the United States National Kidney Foundation guidelines, respectively.
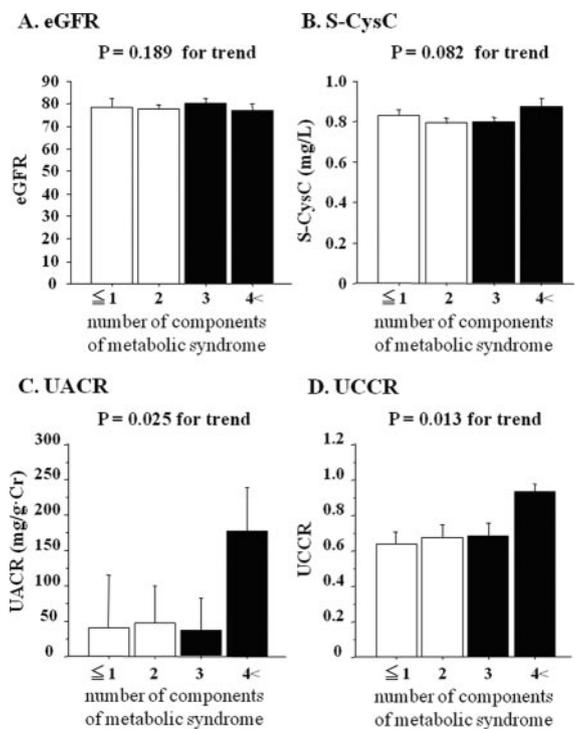

Figure 2. | **The values of eGFR (A), S-CysC (B), UACR (C), and UCCR (D) stratified according to the number of components of metabolic syndrome after gender and age adjustment.** The data are shown as the means ± SEM. The differences between groups were assessed by ANCOVA. Nonmetabolic syndrome and metabolic syndrome are indicated by the open and closed bars, respectively.

Similarly, U-B2MG and U-NAG were significantly correlated with the number of MS risk factors ($P < 0.05$) (Supplemental Figure IC and ID). The mean levels of U-B2MG were increased in accordance with the prognostic stage of CKD (Supplemental Figure IA), whereas U-NAG was not significantly correlated with decreased eGFR (Supplemental Figure IB).

Effect of Body Weight-reduction therapy on Renal Markers and Metabolic Parameters

Of the 182 patients, 54 (30%) successfully reduced their body weight by more than 5%. In those who were successful, BMI, waist circumference, SBP, FPG, HbA1c, LDL-C, and CAVI were significantly decreased, and adiponectin was significantly increased 3 months after the weight-reduction therapy ($P < 0.05$) (Table 3). Among all of the renal markers examined, only UCCR was decreased markedly after successful weight reduction ($P < 0.01$). There were no significant reductions in eGFR, S-CysC, UACR, U-B2MG, or U-NAG. Of those unsuccessful in body-weight reduction ($n = 128$), none of the above parameters changed throughout the study (Table 3). The decrease in logU-CCR was strongly correlated with those in BMI, HbA1c, and CAVI ($r = 0.221, 0.159,$ and $0.198,$ respectively; $P < 0.05$) (Supplemental Table 3). In this study, multivariate regression analysis revealed that the decrease in BMI was an independent determinant of the

Table 3. Effects of weight reduction in obese patients on metabolic variables and renal markers

	Group Unsuccessful in Weight Reduction		Group Successful in Weight Reduction	
	Before	3 Months	Before	3 Months
Male/female	56/72		25/29	
Age (years)	53.4 ± 1.2		50.4 ± 2.1	
BMI (kg/m^2)	30.7 ± 0.5	30.7 ± 0.5	32.1 ± 0.7	29.7 ± 0.7[a]
Waist circumference (cm)	99.5 ± 1.3	99.1 ± 1.2	102 ± 1.6	96.3 ± 2.2[a]
Systolic blood pressure (mmHg)	140 ± 1.5	137 ± 1.4	137 ± 2.7	130 ± 2.1[a]
Diastolic blood pressure (mmHg)	81.2 ± 1.1	80.7 ± 1.0	81.4 ± 2.5	76.3 ± 1.3
Fasting plasma glucose (mg/dl)	135 ± 4.4	134 ± 4.3	142 ± 7.6	129 ± 7.3[b]
HbA1c (%)	6.85 ± 0.1	6.83 ± 0.1	6.98 ± 0.2	6.45 ± 0.2[a]
IRI (mU/ml)	18.2 ± 1.8	20.2 ± 2.7	22.3 ± 5.7	21.6 ± 10
Triglyceride (mg/dl)	162 ± 8.2	161 ± 6.8	170 ± 12	153 ± 12
HDL-C (mg/dl)	55.1 ± 1.2	53.3 ± 1.0	54.0 ± 1.8	53.5 ± 1.7
LDL-C (mg/dl)	122 ± 3.0	123 ± 0.5	123 ± 4.3	113 ± 3.5[a]
Leptin (ng/ml)	13.9 ± 1.1	14.6 ± 1.0	13.6 ± 2.0	12.2 ± 2.3
Adiponectin (μg/ml)	7.11 ± 0.4	7.73 ± 0.5	6.76 ± 0.5	7.98 ± 0.5[b]
CRP (μg/ml)	1.39 ± 0.2	1.17 ± 0.1	1.32 ± 0.2	1.00 ± 0.1
eGFR	72.2 ± 2.7	70.6 ± 2.3	71.6 ± 3.5	72.1 ± 3.7
S-CysC (mg/L)	0.84 ± 0.03	0.85 ± 0.03	0.81 ± 0.04	0.84 ± 0.05
UACR (mg/g·Cr)	116 ± 45	132 ± 50	158 ± 72	130 ± 58
UCCR	0.78 ± 0.11	0.70 ± 0.08	0.83 ± 0.17	0.66 ± 0.16[a]
U-B2MG (μg/L)	165 ± 36	229 ± 92	184 ± 68	129 ± 39
U-NAG (U/L)	4.70 ± 0.5	4.02 ± 0.6	4.08 ± 1.1	2.96 ± 0.4
CAVI	7.92 ± 0.1	7.89 ± 0.2	7.75 ± 0.3	7.42 ± 0.3[a]

The data are expressed as the means ± SEM.
[a]$P < 0.01$ *versus* before determined by a two-tailed paired t test.
[b]$P < 0.05$ *versus* before determined by a two-tailed paired t test.

reduction in logUCCR during weight-reduction therapy ($\beta = 0.221$, $P = 0.003$) (Supplemental Table 3).

Discussion

Here we demonstrated for the first time that U-CysC is significantly correlated with the number of components of MS, the prognostic stages of CKD, and arterial stiffness. This study also revealed that among renal markers, only UCCR was significantly reduced, and its reduction was correlated with only BMI change during weight-reduction therapy, suggesting that U-CysC is closely associated with obesity itself. Because CysC is expressed ubiquitously, filtrated freely by the glomeruli, and reabsorbed by the proximal tubules, U-CysC has been used as a biomarker reflecting renal tubular dysfunction (17–19). Evidence has accumulated suggesting that not only renal glomeruli and but also renal tubules play an important role in the pathophysiology of diabetic and obesity-related nephropathies (30). Moreover, the rate of renal dysfunction is correlated with the degree of renal tubulointerstitial fibrosis (31). This study highlights the possible role of renal tubular dysfunction in obesity and MS (obesity traits in particular), thereby suggesting that U-CysC could serve as a potential risk marker

that reflects the progression of CVD and CKD, similarly to U-Alb, in patients with obesity and MS.

However, from the present association results of UCCR in comparison with UACR, the superior effect of U-CysC on microalbuminuria is debatable. Microalbuminuria predicts GFR decline, cardiovascular events, and mortality in both the general population and patients with diabetes or hypertension (11,12). In this study, we found that UACR is negatively correlated with eGFR and positively correlated with MS traits and arterial stiffness. Similar to UACR, UCCR was also significantly correlated with several CKD and CVD risk factors, such as eGFR, MS traits, and arterial stiffness. In addition, both UACR and UCCR were significantly and independently correlated with age and HbA1c. During weight-reduction therapy, the reduction of UCCR was also significantly correlated with that of HbA1c, suggesting that increased UCCR associates with hyperglycemia, similarly to UACR, and serves as an index of diabetic nephropathy. On the other hand, only UACR, but not UCCR, was correlated with SBP. It is noteworthy that urinary excretion of CysC and albumin is increased via distinct mechanisms; U-CysC is regulated mainly by renal tubular dysfunction, and urinary albumin excre-

tion is regulated mainly by glomerular leakage (11,17,18). The differential correlations of UCCR and UACR with clinical parameters may be attributed to the different mechanisms of their production. Leptin was found to be independently associated with UCCR on multivariate analysis, suggesting that UCCR may be more closely related to obesity itself and obesity-related nephropathy than UACR. It may be clinically beneficial to investigate the different implications and specificities of U-CysC and U-Alb and the merits of utilizing them in combination as an index of CVD or CKD in obesity. To clarify the involvement of U-Alb and U-CysC or glomerular and tubular disorders in the progression of CVD and CKD accompanied by obesity and metabolic diseases, the long-term observation of an increased number of patients is necessary.

Evidence has suggested that S-CysC is associated with GFR reduction and arterial stiffness in patients with conventional CVD risk factors (15,16,32,33). We also found that S-CysC is significantly correlated with the prognostic stages of CKD. However, there was no significant correlation between S-CysC and the number of MS components or age-adjusted CAVI in obesity. It is conceivable that increased U-CysC affects S-CysC in obesity, thereby decreasing the sensitivity for predicting CVD risk factors. Therefore, our data suggest that U-CysC might reflect the atherogenic CVD risk factors relative to S-CysC in obese patients. Further studies are needed to explore the homeostasis of CysC in obesity.

U-B2MG and U-NAG are established biomarkers for renal tubular dysfunction. Recent evidence has also suggested that U-B2MG and U-NAG are associated with CVD risks in type 2 diabetic patients (21,34). We also observed that U-B2MG and U-NAG are significantly correlated with MS traits and that U-B2MG is significantly correlated with eGFR decline. However, only U-CysC, but not U-B2MG and U-NAG, was significantly associated with age-adjusted CAVI, an index of arterial stiffness, in obese patients. These observations could be attributed to the following reasons: (1) U-CysC has different kinetics from U-B2MG and U-NAG, being more closely correlated with creatinine (17,19); (2) the major site of their production differs from one another (for instance, proximal renal tubules for NAG, lymphatic tissues for B2MG, and systemic nucleated cells for CysC) (35–37); and (3) each has distinct functional properties (for instance, NAG as a glycolytic enzyme and CysC as a cysteine proteinase inhibitor). Because cysteine proteinase inhibitors are involved in atherogenesis (35–37), it would be interesting to examine the role of CysC in the pathophysiology of atherosclerosis.

Weight reduction is essential for the management of obesity-related complications (24–26). It has been reported that moderate weight loss effectively ameliorates proteinuria and GFR decline in obesity and MS (24–26). The data of this study demonstrate that successful weight reduction reduces UCCR, suggesting that renal tubular damage in obese patients is reversibly improved by weight reduction. In this regard, Chagnac et al. (38) reported that weight reduction improves glomerular hyperfiltration and ameliorates obesity-related glumerulopathy. We and others previously demonstrated that weight reduction alleviates hyperglycemia, hypertension, hyperlipidemia, and arterial stiffness in patients with obesity and MS (22,23,39). Our data, therefore, newly support the concept that weight-reduction therapy is effective to improve obesity-related tubular dysfunction and subsequently CKD.

The dysregulation of adipocytokine production may contribute to the pathophysiology of metabolic syndrome (40,41). In this study, we demonstrated that leptin is positively correlated with UCCR in obese patients. Leptin, which is overproduced during the course of obesity, is reported to accelerate atherosclerotic lesions, glomerulosclerosis, and renal tubulointerstitial fibrosis in rodent models (42–45). It is conceivable that the production of pro-inflammatory adipocytokine accelerates renal tubular damage as well as arterial stiffness in obese patients. On the other hand, it has recently been reported that the plasma concentration of adiponectin, an anti-inflammatory adipocytokine (46), is elevated because of reduced clearance in chronic renal disorders (47), which might be related to our finding of a positive correlation between the adiponectin and UCCR. The pathophysiological significance of U-CysC in obesity-associated CKD onset and advancement may be clarified through further investigations of how adipocytokines are involved in CysC dynamics in obesity.

There are some study limitations to our study. The study had a cross-sectional design and a short-term intervention and did not examine CVD-related morbidity or mortality in this cohort. Although many prospective studies have shown CRP and arterial stiffness to be independent predictors of future CVD events (48,49), they cannot reflect the critical hard end points of CVD and/or CKD. Accordingly, to clarify the significance of U-CysC as a risk factor for CVD and CKD, it is necessary to conduct a long-term prospective cohort study to examine CVD- and CKD-related morbidity and mortality along with CRP and CAVI. We acknowledge that the correlations between U-CysC and various parameters were not strong, so repeated studies with larger sample sizes should be performed. Furthermore, this study examined the usefulness of U-CysC by classifying it according to the presence or absence of MS. Because there are various opinions on whether the concept of MS can fully contribute to CVD management (50), the clinical implications of these findings should be studied from various viewpoints in future.

In conclusion, this study is the first to demonstrate that increased UCCR is significantly associated with the severity of CKD and MS and arterial stiffness, CVD risk factors, in obese patients. Our data also suggest that U-CysC is helpful for assessing the effect of weight reduction on CVD and CKD risk factors in obesity. Therefore, U-CysC could

serve as a potential biomarker for evaluating and managing the progression of CVD and CKD in patients with obesity and MS.

Disclosures
None.

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