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ORIGINAL ARTICLE

Performance of serum cystatin C versus serum creatinine as a marker of glomerular filtration rate as measured by inulin renal clearance

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Abstract
Introduction Serum cystatin C was recently proposed as an alternative marker of glomerular filtration rate (GFR), with a suggested better performance than creatinine. However, detailed studies are limited. We evaluated the performance of cystatin C as a GFR marker.
Methods GFR was measured by inulin clearance in 763 Japanese subjects. Factors other than GFR influencing serum cystatin C or serum creatinine were analyzed by multivariate analyses.
Results After adjustment for GFR, the value of serum creatinine was 25.2% lower in females than males, and decreased by 5.2% for every 20 years of age. Serum cystatin C was 8.2% lower in females, and did not change significantly with aging. Creatinine but not cystatin C was significantly affected by body weight, height and body mass index after adjustment for GFR, gender and age. The correlation coefficient between GFR and 1/cystatin C was significantly higher than that of 1/creatinine in total subjects (0.866 and 0.810, respectively, $p < 0.001$). Unlike serum creatinine, serum cystatin C did not increase in association with the reduction of GFR in subjects with very low GFR. The regression line of 1/cystatin C against GFR showed a significantly negative intercept of about -8 ml/min/1.73 m^2.
Conclusion The performance of serum cystatin C was not good in the subjects with very low GFR. Non-renal elimination of cystatin C may contribute to the result. The correlation between reciprocal cystatin C and GFR suggested its superiority in predicting GFR compared to creatinine in subjects with normal and mildly reduced GFR.

Keywords Creatinine · Cystatin C · Inulin clearance · Glomerular filtration rate

Introduction

Serum creatinine level has been used to assess renal function, but is often affected by muscle mass, which is dependent on age, weight, and gender [1]. Serum cystatin C was recently proposed as an alternative marker of glomerular filtration rate (GFR), and its higher performance compared with creatinine has been suggested from a meta-analysis [2]. However, detailed studies on the comparison between cystatin C and serum creatinine as markers of GFR are limited. In the present study, we compared the performance of serum cystatin C and serum creatinine as a GFR marker in subjects stratified by gender and age. We confirmed the better performance of cystatin C but also found an apparent non-renal elimination of cystatin C in subjects with low GFR that may affect the performance of cystatin C as a GFR marker.

On behalf of the collaborators for developing Japanese equation for estimating GFR.

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Methods

Subjects and measurements

To compare the performance of serum cystatin C with that of serum creatinine, we used same data set from which the GFR equation for Japanese was developed. Details of the subjects have been reported previously [3, 4]. A total of 763 Japanese patients in 80 medical centers were included. We stratified the subjects according to gender and into three age groups (18–39, 40–59 and 60–79 years old). GFR was measured by inulin renal clearance [3], and serum creatinine was measured by the IDMS-traceable enzymatic method [3]. In the present study, we analyzed the serum cystatin C values which were measured with serum creatinine previously in a single laboratory. Cystatin C was measured by nephrometric assay (Dade Behring).

Multivariate analyses

Factors other than GFR influencing serum cystatin C levels were analyzed by multivariate linear regression analyses. Cystatin C levels and GFR were log-transformed. Age, height, weight and body mass index (BMI) were used as the raw data. Gender was expressed as a binary factor. After adjusting for GFR, the percent change in serum level of cystatin C for a change of 20 years of age, female gender and one unit in the variables such as height, weight, and BMI was analyzed. We also examined the same analyses using serum creatinine levels.

Correlation coefficient between GFR and 1/cystatin C

The correlation coefficient between GFR and 1/cystatin C was analyzed in total subjects and in subjects stratified by gender and age groups (18–39, 40–59 and 60–79 years old). To evaluate an apparent non-renal elimination of cystatin C, the intercept of the linear regression line in the reciprocal plot was calculated. The same analyses were performed for reciprocal serum creatinine. The relationship between GFR and reciprocal creatinine curved upwards slightly, and therefore linear regression lines for 1/creatinine and 1/cystatin C were separately calculated in subjects with serum levels higher than 1.5 mg/dl and 1.5 mg/L, respectively.

Statistical analysis

Data were expressed as means ± SD. $p < 0.05$ was considered statistically significant. Statview version 4.02 (SAS Institute) and JMP 8.01 (SAS Institute) were used for statistical analyses. Smoothed lines fit to the data (Figs. 2, 3, 5) were calculated using spline model of JMP 8.01 (SAS Institute).

Results

Table 1 shows the characteristics of the study subjects. Mean measured GFR of males was significantly lower than that of females (54 ± 34 and 65 ± 36 ml/min/1.73 m^2, respectively). Mean measured GFR in the older age group was significantly lower than that in the younger age group in both males and females.

Multivariate analyses

Serum creatinine was 25.2% lower in females than in males, and decreased by 5.2% for every 20 years of age after adjustment for GFR (Table 2). Serum creatinine was significantly increased in association with increase in body weight, height, and BMI after adjustment for GFR, gender and age. Serum cystatin C was 8.2% lower in females than in males, and was not significantly changed by age after adjustment for GFR. Cystatin C was not significantly

Table 1 Characteristics of the study subjects

	N	Age	GFR (ml/min/1.73 m^2)	Cystatin-C (mg/L)	Creatinine (mg/dl)	Weight (kg)	Height (cm)
Male							
18–39 years old	119	30 ± 6	77 ± 37	1.34 ± 0.89	1.41 ± 1.32	69 ± 13	171 ± 5
40–59 years old	145	52 ± 6	54 ± 31	1.63 ± 1.02	1.81 ± 1.68	68 ± 11	169 ± 7
60–79 years old	189	68 ± 5	41 ± 26	2.01 ± 1.04	2.17 ± 1.73	63 ± 11	164 ± 7
Total	465	54 ± 17	54 ± 34	1.72 ± 1.04	1.87 ± 1.64	66 ± 12	167 ± 7
Female							
18–39 years old	91	30 ± 6	83 ± 33	1.07 ± 0.81	0.91 ± 0.96	53 ± 8	159 ± 6
40–59 years old	106	51 ± 5	64 ± 37	1.41 ± 0.95	1.22 ± 1.22	54 ± 12	155 ± 5
60–79 years old	94	68 ± 5	50 ± 28	1.66 ± 1.12	1.37 ± 1.17	51 ± 8	151 ± 6
Total	298	51 ± 17	65 ± 36	1.40 ± 0.99	1.18 ± 1.13	53 ± 10	155 ± 6

Table 2 Factors affecting serum cystatin C and creatinine after adjustment for GFR

Variable	Cystatin C % change (95% CI)	*p*	Creatinine % change (95% CI)	*p*
Adjusted for GFR				
Age (20 years)	−0.1 (−2.0 to 2.2)	0.9	−5.2 (−7.9 to −2.5)	0.0002
Female	−8.2 (−11.2 to −5.2)	<0.0001	−25.2 (−28.1 to −22.4)	<0.0001
Height (cm)	0.4 (0.2 to 0.6)	<0.0001	1.5 (1.3 to 1.7)	<0.0001
Weight (kg)	0.2 (0.0 to 0.3)	0.006	0.9 (0.7 to 1.0)	<0.0001
BMI (kg/m^2)	0.2 (−0.3 to 0.6)	0.5	1.4 (0.9 to 2.0)	<0.0001
Adjusted for GFR, age and gender				
Height (cm)	0.2 (−0.1 to 0.5)	0.1	0.6 (0.3 to 0.9)	0.0001
Weight (kg)	0.0 (−0.1 to 0.2)	0.8	0.4 (0.2 to 0.6)	<0.0001
BMI (kg/m^2)	−0.1 (−0.5 to 0.4)	0.8	0.8 (0.3 to 1.3)	0.001

% change percent change in serum level of creatinine or cystatin C for a change of 20 years of age, female gender and one unit in the variable (height, weight and BMI)

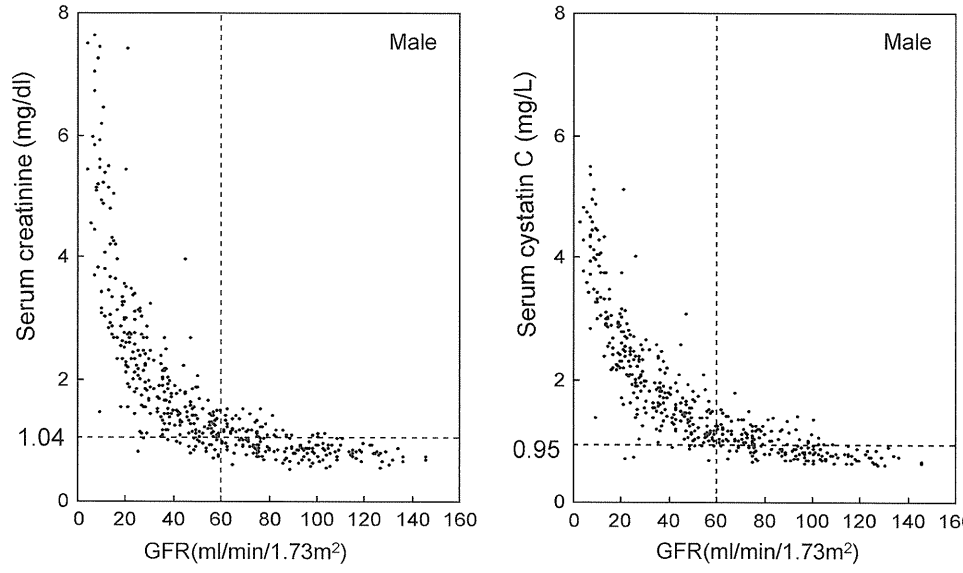

Fig. 1 Relationship between GFR and serum concentration of creatinine or cystatin C. *Left* GFR versus serum concentration of creatinine in male subjects. *Dotted lines* show upper reference limit of serum creatinine and lower reference limit of GFR (1.04 mg/dl and 60 ml/min/1.73 m^2, respectively). *Right* GFR versus serum concentration of cystatin C in male subjects. *Dotted lines* show upper limit of serum cystatin C and lower reference limit of GFR (0.95 mg/dl and 60 ml/min/1.73 m^2, respectively)

changed by body weight, height and BMI after adjustment for GFR, gender and age.

Relationship between GFR and serum concentration of cystatin C

The relationship between GFR and serum concentrations of cystatin C is shown in Fig. 1. The plot of serum cystatin C showed a curvilinear pattern that was similar to that of serum creatinine. The relationship between GFR and 1/cystatin C was almost linear, while the plots of 1/creatinine seemed to be curving upwards slightly (Figs. 2, 3). The correlation coefficient in the reciprocal plot of cystatin C was significantly higher compared with that of creatinine in total subjects ($r = 0.866$ and 0.810, respectively) (Table 3). The correlation coefficients of cystatin C were consistently higher than the values of creatinine in subjects stratified by gender and three age groups, although statistically not significant.

Relationship between serum concentration of creatinine and serum concentration of cystatin C

The relationship between serum concentration of creatinine and serum concentration of cystatin C was not linear (Fig. 4). Unlike serum creatinine, serum cystatin C did not increase in association with reduction of GFR in subjects with GFR below 15 ml/min/1.73 m^2 (Fig. 5). To evaluate the apparent non-renal elimination of cystatin C, the linear regression line of the reciprocal plots was calculated in subjects with serum cystatin C higher than 1.5 mg/L. Intercepts (95% confidence interval [CI]) of the regression lines in male and female were −8.4 (−12.1, −4.8) ml/min/1.73 m^2 and −9.1 (−15.5, −2.6) ml/min/1.73 m^2, respectively (Table 4). The values were significantly lower than zero ($p < 0.01$). The linear regression line of the reciprocal plots of creatinine that was calculated in subjects with serum creatinine higher than 1.5 mg/dl intersected near the origin. Intercepts (95% CI) of the regression lines in

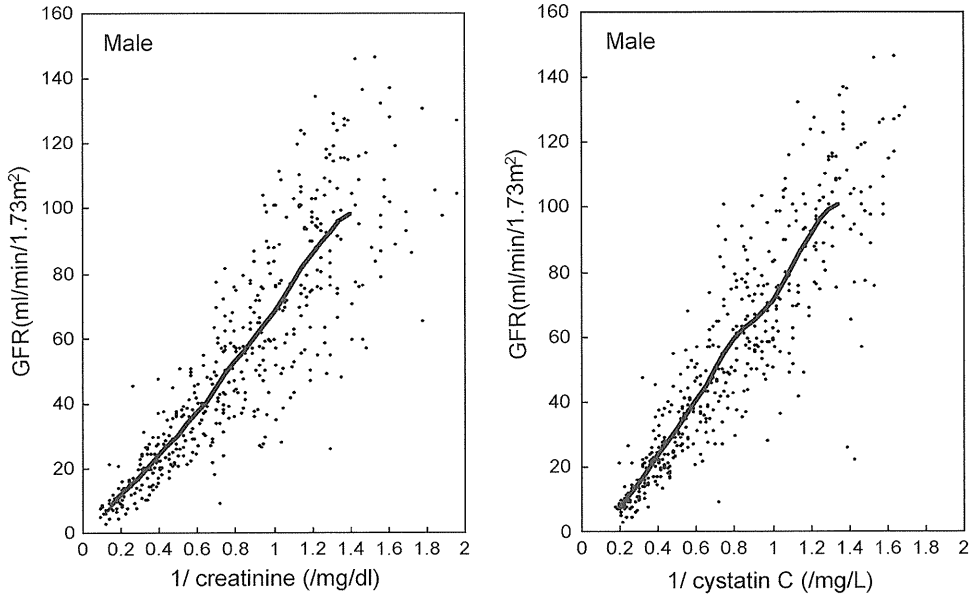

Fig. 2 Reciprocal plots of creatinine and cystatin C in male subjects. *Left* GFR versus 1/creatinine. *Right* GFR versus 1/cystatin C. *Smooth lines* show the fit of the data

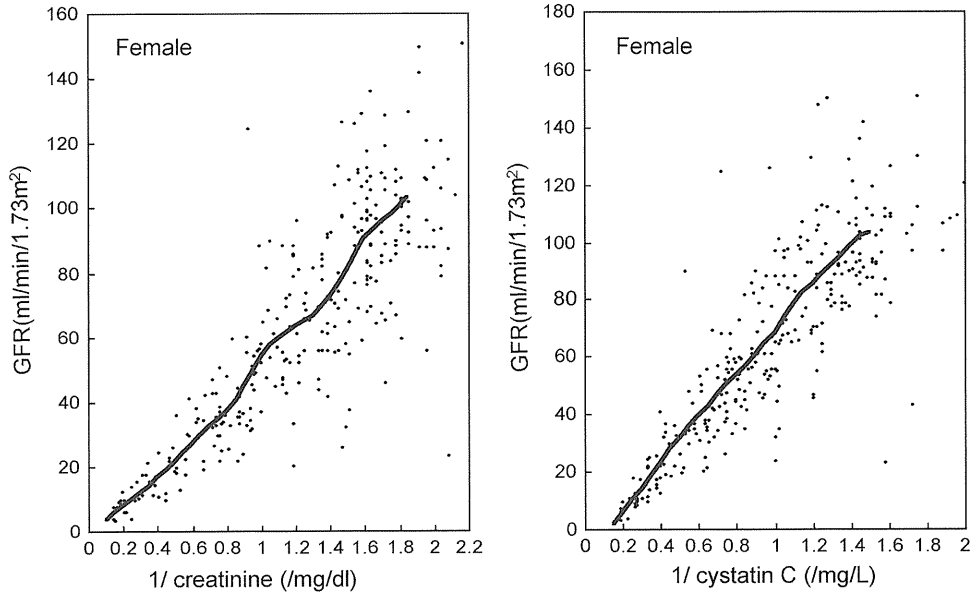

Fig. 3 Reciprocal plots of creatinine and cystatin C in female subjects. *Left* GFR versus 1/creatinine. *Right* GFR versus 1/cystatin C. *Smooth lines* show the fit of the data

males and females were −0.9 (−3.8, 2.0) ml/min/1.73 m^2 and −2.2 (−6.5, 2.1) ml/min/1.73 m^2, respectively. The values were not significantly different from zero.

Discussion

Serum creatinine and cystatin C are well-known markers of GFR. However, few studies have investigated their comparative performance relative to GFR measured by inulin renal clearance. In present study, we observed a significant difference in performance of creatinine and cystatin C as GFR markers. Gender and age effects were more prominent in creatinine. Serum creatinine was 25.2% lower in females, and declined by 5.2% for every 20 years of age, while serum cystatin C was 8.2% lower in females, and was not significantly associated with age. These results are almost consistent with the reports of Stevens et al. and Knight et al. [5, 6]. Stevens et al. reported that older age was associated with lower serum cystatin C level after adjustment of GFR measured by iothalamate clearance [5]. On the other hand, Knight et al. reported that older age was associated with higher serum cystatin C level after adjusting for creatinine clearance [6]. The backgrounds of the study population such as ethnicity, renal function and physique were different between the studies. The variable factors and methods of GFR measurement may influence the results of the studies.

Table 3 Correlation coefficients between GFR and 1/cystatin C or 1/creatinine in subjects stratified by gender and age groups

	N	GFR (ml/min/1.73 m^2)	Correlation coefficient		*p*
			1/cystatin C	1/creatinine	
Male					
18–39 years old	119	77 ± 37	0.919	0.880	0.11
40–59 years old	145	54 ± 31	0.848	0.833	0.67
60–79 years old	189	41 ± 26	0.865	0.837	0.32
Total	465	54 ± 34	0.890	0.854	0.09
Female					
18–39 years old	91	83 ± 33	0.797	0.786	0.84
40–59 years old	106	64 ± 37	0.815	0.750	0.22
60–79 years old	94	50 ± 28	0.856	0.846	0.80
Total	298	65 ± 36	0.832	0.794	0.17
Male + female					
Total	763	58 ± 35	0.866	0.810	0.0002

p values: difference between cystatin C and creatinine

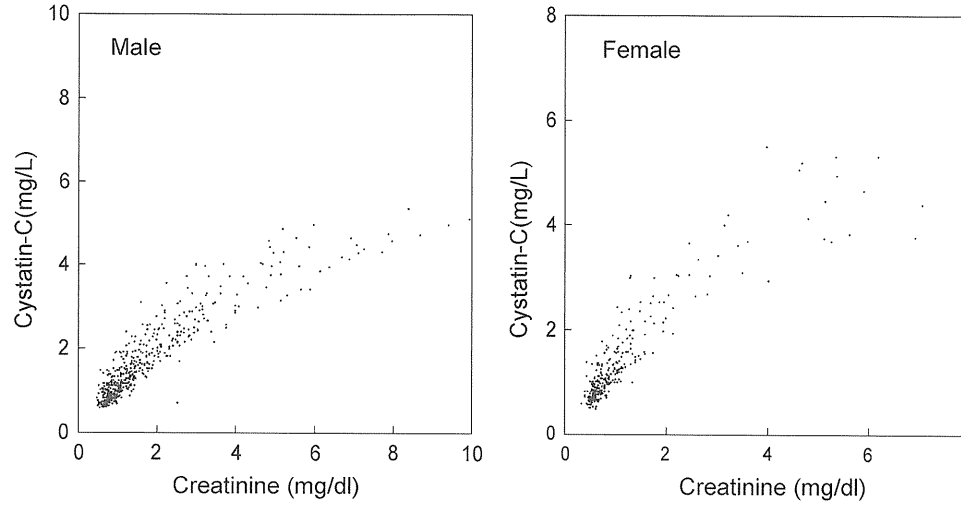

Fig. 4 Relationship between serum concentration of creatinine and serum concentration of cystatin C

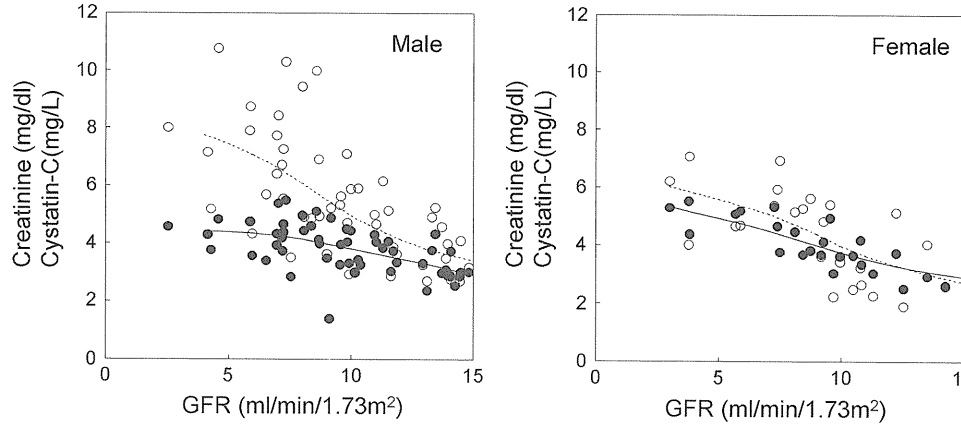

Fig. 5 Serum concentrations of creatinine and cystatin C in subjects with GFR under 15 ml/min/1.73 m^2. Unlike serum creatinine (*open circle*), serum cystatin C (*closed circle*) did not increase as much in association with reduction of GFR in subjects with GFR under 15 ml/min/1.73 m^2. *Smooth lines* show the fit of the data

Generation of creatinine is affected by muscle mass, which is dependent on physique such as weight and height. Higher serum creatinine was associated with higher body weight, height, and BMI after adjustment for GFR, gender and age (Table 2). The association of these parameters with serum cystatin C level was much smaller than with creatinine. These results are consistent with the report of Stevens et al. [5] except for BMI. They reported that the

Table 4 Intercept of the regression line between GFR and 1/cystatin C or 1/creatinine in subjects stratified by gender and age groups

	1/cystatin C			1/creatinine		
	N	Intercept (95% CI)	*p*	*N*	Intercept (95% CI)	*p*
Male						
18–39 years old	34	−11.2 (−19.7 to −2.7)	0.01	30	−0.1 (−8.9 to 8.7)	0.9
40–59 years old	50	−7.4 (−15.1 to 0.3)	0.06	57	−0.5 (−5.3 to 6.2)	0.9
60–79 years old	118	−9.5 (−14.5 to −4.6)	0.0002	100	−1.8 (−5.6 to 2.0)	0.3
Total	222	−8.4 (−12.1 to −4.8)	<0.0001	194	−0.9 (−3.8 to 2.0)	0.5
Female						
18–39 years old	9	−8.3 (−18.0 to 1.3)	0.08	6	−2.7 (−15.8 to 10.4)	0.6
40–59 years old	35	−10.6 (−20.6 to −5.2)	0.04	18	−1.3 (−7.0 to 4.5)	0.6
60–79 years old	37	−7.6 (−19.9 to 4.8)	0.2	25	−2.1 (−10.1 to 6.0)	0.6
Total	87	−9.1 (−15.5 to −2.6)	0.007	51	−2.2 (−6.5 to 2.1)	0.3

Regression lines were made using subjects with serum cystatin C over 1.5 mg/L or serum creatinine over 1.5 mg/dl

Table 5 Simulation models for reciprocal plots of creatinine and cystatin C

	Model A			Model B			Model C	
GFR	CCR/GFR	G1	P1	N-CL	G2	P2	G2	P2
100	1.20	0.96	0.80	8	0.096	0.80	0.0800	0.80
90	1.25	0.96	0.85	8	0.096	0.88	0.0793	0.88
80	1.30	0.96	0.92	8	0.096	0.98	0.0785	0.98
70	1.35	0.96	1.02	8	0.096	1.11	0.0775	1.11
60	1.40	0.96	1.14	8	0.096	1.27	0.0762	1.27
50	1.45	0.96	1.32	8	0.096	1.49	0.0745	1.49
40	1.50	0.96	1.60	8	0.096	1.80	0.0720	1.80
30	1.55	0.96	2.06	8	0.096	2.27	0.0682	2.27
20	1.60	0.96	3.00	8	0.096	3.09	0.0617	3.09
10	1.65	0.96	5.82	8	0.096	4.80	0.0480	4.80

GFR glomerular filtration rate (ml/min), *Model A* simulation model for serum creatinine, *CCR/GFR* CCR/GFR ratio, *G1* generation of creatinine (mg/min), *P1* plasma concentration of creatinine (mg/dl), *Model B* and *Model C* simulation models for serum cystatin C, *N-CL* non-renal clearance of cystatin C (ml/min), *G2* generation of cystatin C (mg/min), *P2* plasma concentration of cystatin C (mg/L)

percent change of cystatin C was higher than the value of creatinine according to the change in BMI, and speculated an association between fat mass and cystatin C levels. The mean and interquartile range of BMI in the study by Stevens et al. were 27.7 and 7.3 kg/m^2, while the values in our study were much lower (22.9 and 4.8 kg/m^2, respectively). The lower prevalence of obesity in our subjects may contribute to the inconsistent results regarding the association with BMI.

The relationship between GFR and 1/cystatin C was almost linear, evaluated by the smoothed line fit to the data (Figs. 2, 3). On the other hand, the relationship between GFR and 1/creatinine was slightly upwardly curving. This could be one of the reasons that reduces the performance of creatinine in the correlation analysis. Creatinine is secreted from tubules as well as filtered from glomeruli. Therefore, the creatinine clearance (CCR) exceeds the GFR. About 20% of creatinine is secreted from the tubule in normal subjects [1], and the tubular secretion increases up to about 50% with reduction in GFR [1]. The change in tubular secretion may contribute to the upward curve of the reciprocal plot of creatinine. We made a simulation model in which the CCR/GFR ratio was increased from 1.2 to 1.7 according to the reduction in GFR with constant generation of creatinine (Table 5; Fig. 6, model A). The simulation model confirmed the effect of tubular secretion on the upward curving of the reciprocal plot.

The reciprocal plot of serum cystatin C had a significantly negative intercept, suggesting an apparent non-renal elimination of the marker. Assuming that the generation of cystatin C is independent of GFR, non-renal elimination of cystatin C is estimated as the intercept of the reciprocal plot. We obtained an apparent non-renal clearance of cystatin C of about 8 ml/min/1.73 m^2. Sjostrom et al. [7, 8] reported similar results using the reciprocal plot of cystatin C. They measured GFR by plasma clearance of iohexol and reported the apparent non-renal elimination of cystatin C as 22.3 ml/min/1.73 m^2 in a preliminary

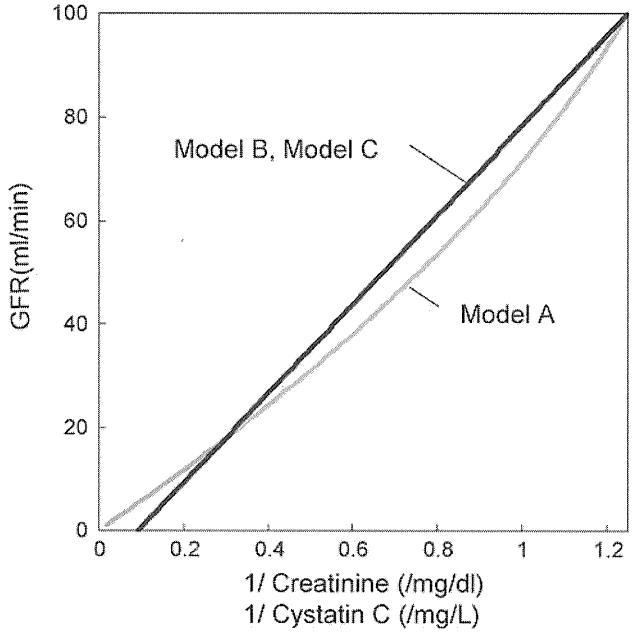

Fig. 6 Simulation models for reciprocal plots of creatinine and cystatin C. Detailed values for simulation models are described in Table 5. Model A is a simulation model for the reciprocal plot of creatinine. CCR/GFR ratio increases according to reduction of GFR. This leads to the upward curve of the reciprocal plot of creatinine. Model B is a simulation model for the reciprocal plot of cystatin C with constant non-renal clearance. This model shows the linear relationship between GFR and 1/cystatin C. Model C is a simulation model for the reciprocal plot of cystatin C without non-renal clearance. Controlled change in the generation of cystatin C is required to keep the linear relationship between GFR and 1/cystatin C

study and 14.1 ml/min/1.73 m^2 in a second study[7, 8]. We made two simulation models to explain the apparent non-renal elimination of cystatin C. The first is a simulation model with constant non-renal clearance set at 8 ml/min. The generation of cystatin C was set as the value at which serum cystatin C was 0.8 mg/L when GFR was 100 ml/min. The simulation model with constant non-renal clearance shows a linear relationship between GFR and reciprocal cystatin C with negative intercept (Table 5; Fig. 6, model B). In the second simulation model, the generation of cystatin C was decreased according to reduction in GFR. The same straight line as seen in model B could be drawn. In this case, generation of cystatin C decreases at an accelerated pace according to reduction of GFR (Table 5; Fig. 6, model C). This suggests that non-renal elimination of cystatin C is a more plausible mechanism than rapid reduction of cystatin C generation. Tenstad et al. [9] investigated renal handling of radiolabeled human cystatin C in rat. A considerable amount of extra-renal plasma clearance of labeled cystatin C was observed in nephrectomized rats, that contributed about 15% of the total plasma clearance of cystatin C. A relatively high uptake of radioactivity was recorded in the spleen. They speculated about the removal of cystatin C in the reticuloendothelial system.

Unlike serum creatinine, serum cystatin C did not increase in association with reduction of GFR in subjects with GFR below 15 ml/min/1.73 m^2. The performance of serum cystatin C was not good in these subjects, and therefore,serum cystatin C should not be used for evaluating GFR in subjects with end-stage renal disease. Apparent non-renal elimination of cystatin C contributed to the low performance of the marker. On the contrary, even if non-renal elimination of cystatin C is present at a comparable level, percentage of non-renal elimination in the total clearance would be small in subjects with normal and mildly reduced GFR, suggesting that the effect of non-renal elimination on serum levels of cystatin C is small and difficult to detect. Although the difference between the correlation coefficients of reciprocal cystatin C and reciprocal creatinine was small, cystatin C had a better correlation with GFR than creatinine in total subjects, suggesting that cystatin C concentration may be better than creatinine at predicting GFR in subjects with normal and mildly reduced GFR.

Eriksen et al. [10] showed that cystatin C is not a better estimator of GFR than creatinine, based on the testing of several GFR equations. They showed a large bias of GFR equations from cystatin C compared with GFR equations from creatinine, such as the CKD–EPI equation in the general population. The authors mentioned that the most important factor of the large bias of the cystatin C equations was probably that GFR equations from cystatin C were all developed in populations with CKD and low GFR. The influence of non-GFR factors on plasma cystatin C may differ between these patients and the general population. Also, standardization between assays and laboratories is lacking for cystatin C.

We agree with the comments of Eriksen et al. When the equation from cystatin C was developed from subjects with lower GFR, non-GFR elimination of cystatin C may influence the performance of the equation in subjects with normal or mildly reduced GFR. We have to study the model for GFR estimation including factors such as non-renal clearance. The measurement of cystatin C has not been standardized. There is up to 20% difference in the cystatin C values among various reagent companies [11, 12]. When the cystatin C value has a 20% positive bias compared with the value that was used for development of a GFR estimation equation, the measurement bias leads to about 20% underestimation of GFR and lower performance of the equation. The standardization of the measurement is a fundamental problem of the accuracy of the GFR equations derived from cystatin C. Standardization of the measurement of cystatin C is nowin progress worldwide [13], after which, development and validation of GFR equations from cystatin C will be required.

There are several limitations. The study subjects were almost all patients with native kidney disease, so it may not be possible to generalize the results to healthy subjects. It has been reported that cystatin C levels were influenced by factors other than GFR, such as thyroid function [14, 15], inflammation [5, 6], smoking [6, 8] and immunosuppressive therapy [16]. Multivariate analysis was not adjusted for the above factors in the present study.

Conclusion

The performance of serum cystatin C was not good in subjects with very low GFR; non-renal elimination of cystatin C may contribute to the result. Reciprocal cystatin C had a better correlation with GFR than creatinine in total subjects. Age, gender, body weight, height and BMI had a much smaller effect on cystatin C level than creatinine. These results suggested the superiority of cystatin C over creatinine in predicting GFR in subjects with normal and mildly reduced GFR.

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Appendix

The following investigators participated in the project for developing the Japanese equation for estimating GFR: Asahikawa Medical University; Kenjiro Kikuchi, Masakazu Haneda: Hokkaido University Hospital; Seiji Hashimoto: Sapporo Medical University; Nobuyuki Ura: Tohoku University Hospital; Sadayoshi Ito, Hiroshi Sato: Fukushima Medical University; Tsuyoshi Watanabe: Jichi Medical University; Yasuhiro Ando, Eiji Kusano: Gunma University; Yoshihisa Nojima: University of Tsukuba; Kunihiro Yamagata, Chie Saitoh: Toride Kyodo General Hospital; Tatsuo Shiigai, Yoshitaka Maeda: Saitama Medical University; Hiromichi Suzuki, Yusuke Watanabe: Saitama Medical University; Tetsuya Mitarai, Hajime Hasegawa: The University of Tokyo; Toshiro Fujita: Jikei University; Tatsuo Hosoya: Nihon University; Koichi Matsumoto, Takayuki Fujita: Tokyo Women's Medical University; Kousaku Nitta: Tokyo Women's Medical University Medical Center East; Tsutomu Sanaka: Tokyo Medical and Dental University; Eiichiro Kanda, Sei Sasaki: Juntendo University; Yasuhiko Tomino: Tokyo Medical University Hospital; Toshiyuki Nakano: Showa University; Tadao Akizawa, Hirokazu Honda: Showa University Fujigaoka Hospital; Yoshihiko Inoue, Ashio Yoshimura: Toranomon Hospital; Hiroshi Tsuji: Toho University; Yasushi Ohashi: Teikyo University; Hideaki Nakajima Shunya Uchida: Kyorin University; Akira Yamada: Yokohama City University; Satoshi Umemura, Mai Yanagi: St. Marianna University; Hiro Yamakawa, Kenjiro Kimura: Niigata University; Shinichi Nishi, Fumitake Gejyo: Hamamatsu University; Akira Hishida, Hideo Yasuda, Tomoyuki Fujikura: Nagoya University; Seiichi Matsuo: Aichi Medical University; Hirokazu Imai, Wataru Kitagawa: Fujita Health University Hospital; Satoshi Sugiyama: Mie University; Shinsuke Nomura, Eiji Ishikawa: Toyama Prefectural Central Hospital; Masahiko Kawabata: Hiroyuki Iida: Kanazawa University Hospital; Takashi Wada: Kanazawa Medical University; Hitoshi Yokoyama: University of Fukui Hospital; Haruyoshi Yoshida: Shiga University of Medical Science; Takashi Uzu, Masayoshi Sakaguchi: Kyoto University Hospital, Atsushi Fukatsu: Osaka University; Yasuyuki Nagasawa, Shiro Takahara: Osaka City University; Eiji Ishimura, Yoshiki Nishizawa: Kobe University; Masafumi Fukagawa, Michio Umezu: Hyogo College of Medicine; Nakanishi Takeshi, Izumi Masaaki: Okayama University, Hitoshi Sugiyama, Hirofumi Makino: Kawasaki Medical School; Naoki Kashihara, Tamaki Sasaki: Hiroshima University; Noriaki Yorioka: University of Tokushima; Toshio Doi: Kagawa University; Masakazu Kono: Ehime University; Takafumi Okura: Kyushu University Hospital; Kazuhiko Tsuruya, Akiko Ono: Fukuoka University; Takao Saito, Yasuhiro Abe: Nagasaki University; Akira Furusu: Kurume University; Seiya Okuda: Kumamoto University; Kimio Tomita: University of Miyazaki; Shouichi Fujimoto: University Hospital of The Ryukyus; Iseki Kunitoshi, Schuichi Takishita: Koto Hospital; Isao Ebihara, Yuko Shima: Nara Medical University; Koji Harada, Yoshihiko Saito: Dokkyo Medical University; Atsushi Numabe, Toshihiko Ishimitsu: Kitano Hospital; Eri Muso, Toshiyuki Komiya: Musashino Red Cross Hospital; Ryoichi Ando: Toyonaka Municipal Hospital; Megumu Fukunaga: Ohmihachiman Community Medical Center; Tsuguru Hatta: Sendai Shakaihoken Hospital; Osamu Hota: Tosei General Hospital; Inaguma Daijo: Matsuyama Clinic; Kazuhiro Matsuyama: Rokko Island Hospital; Naoyuki Nakano: Shuwa General Hospital; Masashi Inoshita: Kanagawa Rehabilitation Hospital; Masahisa Kusaka, Masato Mizuguchi.

References

1. Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. Annu Rev Med. 1988;39:465–90.
2. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis. 2002;40:221–6.
3. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, on behalf of the collaborators developing the Japanese equation for

estimating GFR, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
4. Horio M, Imai E, Yasuda Y, Watanabe T, Matsuo S. Modification of the CKD epidemiology collaboration (CKD-EPI) equation for Japanese: accuracy and use for population estimates. Am J Kidney Dis. 2010;56:32–8.
5. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney Int. 2009;75:652–60.
6. Knight EL, Verhave JC, Spiegelman D, Hillege HL, Zeeuw DD, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int. 2004;65:1416–21.
7. Sjostrom PA, Tidman MA, Jones IL. Determination of the production rate and non-renal clearance of cystatin C and estimation of the glomerular filtration rate from the serum concentration of cystatin C in humans. Scand J Clin Lab Invest. 2005;65:111–24.
8. Sjostrom PA, Jones IL, Tidman MA. Cystatin C as a filtration marker—haemodialysis patients expose its strengths and limitations. Scand J Clin Lab Invest. 2009;69:65–72.
9. Tenstad O, Roald AB, Grubb A, Aukland K. Renal handling of radiolabelled human cystatin C in the rat. Scand J Clin Lab Invest. 1996;56:409–14.
10. Eriksen BO, Mathisen UD, Melsom T, Ingebretsen OC, Jenssen TG, Njolstad I, et al. Cystatin C is not a better estimator of GFR than plasma creatinine in the general population. Kidney Int. 2010;78:1305–11.
11. Levinson S. Calibration of the Siemens cystatin C immunoassay has changed over time. Clin Chem. 2011;57:777–8.
12. Voskoboev NV, Larson TS, Rule AD, Lieske C. Importance of cystatin C assay standardization. Clin Chem. 2011;57:1209–11.
13. Grubb A, Blirup-Jensen S, Lindstrom V, Schmidt C, Althaus H, Zegers I. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. Clin Chem Lab Med. 2010;48:1619–21.
14. Kotajima N, Yanagawa Y, Aoki T, Tsunekawa K, Morimura T, Ogiwara T, et al. Influence of thyroid hormones and transforming growth factor-β1 on cystatin C concentrations. J Int Med Res. 2010;38:1365–73.
15. Fricker M, Wiesli P, Brandle M, Schwegler B, Schmid C. Impact of thyroid dysfunction on serum cystatin C. Kidney Int. 2003;63:1944–7.
16. Rule AD, Bergstralh EJ, Slezak JM, Bergert J, Larson TS. Glomerular filtration rate estimated by cystatin C among different clinical presentations. Kidney Int. 2006;69:399–405.

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Original Article

Decreasing Abdominal Circumference Is Associated with Improving Estimated Glomerular Filtration Rate (eGFR) with Lifestyle Modification in Japanese Men: A Pilot Study

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The link between changes in a subject's metabolic syndrome components and his estimated glomerular filtration rate (eGFR) was evaluated in healthy Japanese men. We used data from 120 Japanese men (45.5 ± 8.4 years) with a 1-year follow up. eGFR was defined by a new equation developed for Japan. There were no significant differences in eGFR between men with and without metabolic syndrome components at baseline. Subjects were given advice for dietary and lifestyle improvement. At the 1-year follow up, almost all metabolic syndrome components were significantly improved. However, eGFR was significantly decreased. The changes in eGFR were weakly correlated with abdominal circumference ($r = -0.232$, $p = 0.0106$). A decrease in abdominal circumference may be associated with improving eGFR in Japanese men.

Key words: abdominal circumference, estimated glomerular filtration rate (eGFR), metabolic syndrome, lifestyle modification

Chronic kidney disease (CKD) has become a public health challenge and is a common disorder [1]. For example, approximately 20% of adults have CKD, which is defined as kidney damage or a glomerular filtration rate (GFR) $< 60\,ml/min/1.73\,m^2$ for at least 3 months, regardless of cause [2]. We have also previously reported in a cross-sectional study that the estimated glomerular filtration rate (eGFR) [3] in men with abdominal obesity and in women with hypertension was significantly lower than that in subjects without these components of metabolic syndrome [4]. In addition, we have shown that decreasing systolic blood pressure is associated with improving eGFR with lifestyle modification in healthy Japanese women [5]. However, whether decreases in metabolic syndrome components are beneficial for improving eGFR, and what effect this has on eGFR remain to be investigated in a longitudinal study in Japanese men.

In this study, we evaluated the link between changes in eGFR and changes in metabolic syndrome components in Japanese men with a 1-year follow up.

Subjects and Methods

Subjects. We used data for 120 Japanese men from a data-base of 16,383 people at the Okayama Southern Institute of Health in Okayama prefecture, Japan, aged 45.5 ± 8.4 years, who met the following criteria: (1) received a health check-up, including

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special health guidance and a follow-up check-up 1-year later; (2) received anthropometric measurements, fasting blood examination, and blood pressure measurements as part of the annual health check-up; (3) received no medications for diabetes, hypertension, and/or dyslipidemia; and (4) provided written informed consent (Table 1).

At the first health check-up, all subjects were given instructions by well-trained medical staff on how to change their lifestyle as special health guidance. Nutritional instruction was provided with a well-trained nutritionist, who planned a diet for each subject based on their data and provided simple instructions (*i.e.* not to eat too much and to consider balance when they eat). Exercise instruction was also provided by a well-trained physical therapist, who encouraged each subject to increase their daily number of steps walked.

Ethical approval for the study was obtained from the Ethical Committee of the Okayama Health Foundation.

Anthropometric and body composition measurements. Anthropometric and body compositions were evaluated based on the following parameters: height, body weight, and abdominal circumference. Body mass index (BMI) was calculated by weight / [height]^2, in kg/m^2. Abdominal circumference was measured at the umbilical level in standing subjects after normal expiration [6].

Blood pressure measurements at rest. Resting systolic and diastolic blood pressures were measured indirectly using a mercury sphygmomanometer placed on the right arm of the seated participant after at least 15 min of rest.

Urine examination. Urine samples were collected from the second-morning urine (before 10 a.m.) and subjected to examination within 1 h. The urine examination was performed using urine test strips (BAYER, Tokyo, Japan). The reagent strip was dipped directly into the urine sample. Just after dipping, the sample was graded as −: negative, ±: trace positive, +: positive (30 mg/dl), 2+: positive (100 mg/dl), 3+: positive (300 mg/dl), or 4+: positive (1,000 mg/dl) by comparison with a standard color chart found on the container's label.

Blood sampling and assays. We measured overnight fasting serum levels of creatinine (Cr) (enzymatic method), high-density lipoprotein (HDL) cholesterol, triglycerides (L Type Wako Triglyceride・H, Wako Chemical, Osaka, Japan), and blood sugar. eGFR was calculated using the following equation: $\text{eGFR (ml/min/1.73 m}^2) = 194 \times \text{Cr}^{-1.094} \times \text{Age}^{-0.287}$ [3]. Reduced eGFR was defined as an eGFR < 60 ml/min/1.73 m^2.

Definition of metabolic syndrome. Men with an abdominal circumference in excess of 85 cm were defined as having metabolic syndrome if they also had two or more of the following components: 1) Dyslipidemia: triglycerides ≥ 150 mg/dl and/or HDL cholesterol < 40 mg/dl, 2) High blood pressure: blood pressure $\geq 130/85$ mmHg, 3) Impaired glucose tolerance: fasting plasma glucose ≥ 110 mg/dl [6].

Table 1 Clinical characteristics and changes in parameters with 1-year follow up

	Baseline	Follow up	*p*
Number of Subjects	120		
Age	45.5 ± 8.4		
Height (cm)	169.0 ± 5.3		
Body weight (kg)	75.6 ± 11.3	74.0 ± 10.7	**<0.0001**
Body mass index (kg/m^2)	26.5 ± 3.6	25.9 ± 3.4	**<0.0001**
Abdominal circumference (cm)	88.5 ± 9.8	86.3 ± 9.2	**<0.0001**
Systolic blood pressre (mmHg)	131.5 ± 14.6	123.9 ± 12.5	**<0.0001**
Diastolic blood pressure (mmHg)	82.6 ± 11.5	77.0 ± 9.2	**<0.0001**
Triglyceride (mg/dl)	153.3 ± 110.2	121.7 ± 80.3	**0.0011**
HDL cholesterol (mg/dl)	54.2 ± 14.6	56.2 ± 14.9	**0.0390**
Blood sugar (mg/dl)	102.7 ± 18.1	104.2 ± 28.3	0.3710
Cr (mg/dl)	0.81 ± 0.12	0.84 ± 0.12	**0.0012**
eGFR (ml/min/1.73 m^2)	84.0 ± 13.9	80.1 ± 13.1	**<0.0001**
		Mean ± SD	

Statistical analysis. Data are expressed as means ± standard deviation (SD). A statistical analysis was performed using a paired t test and χ^2 test: $p < 0.05$ was considered to be statistically significant. Pearson's correlation coefficients were calculated and used to test the significance of the linear relationship among continuous variables.

Results

The clinical parameters at the baseline and the 1-year follow up are summarized in Table 1. Anthropometric and body composition parameters such as body weight, BMI, and abdominal circumference were significantly reduced with lifestyle modification after 1 year. Cr was significantly increased, and eGFR was decreased. Thirty-six subjects were diagnosed as having metabolic syndrome at baseline, and 18 subjects were diagnosed as having metabolic syndrome after 1 year, which was a significant reduction ($p < 0.0001$). Two subjects were diagnosed with reduced eGFR at baseline, and 3 subjects were diagnosed with reduced eGFR at the 1-year follow up. In addition, four subjects were identified as trace positive, 2 were identified as positive (+), and one was identified as positive (2+) for proteinuria at baseline, while 5 were identified as trace positive, 4 as positive (+), and 2 as positive (2+) at the 1-year follow up.

In subjects not taking medications, we also compared eGFR levels between the groups with and without each component of the Japanese definition of metabolic syndrome (Table 2). eGFR in men with abdominal obesity was significantly higher than that in men without abdominal obesity. However, there were no significant differences of eGFR between the groups with or without other components of metabolic syndrome. In addition, eGFR in subjects with metabolic syndrome was similar to that in subjects without it.

We further evaluated the relationship between changes in eGFR and changes in clinical parameters. Changes in eGFR were weakly correlated with changes in abdominal circumference ($r = -0.232$, $p = 0.0106$) (Table 3, Fig. 1). After we excluded one subject with abnormal changes in eGFR (-37.7 ml/min/1.73 m^2), changes in eGFR were still weakly correlated with changes in abdominal circumference ($n = 119$, $r = -0.203$, $p = 0.0265$). However, changes in eGFR were not significantly correlated with changes in other metabolic components.

Finally, we investigated the changes in eGFR amongst men with different levels of increased abdominal circumference [Group I: Delta (delta represents positive changes in abdominal circumference) abdominal circumference ≧ 0 cm, Group D: Delta abdominal circumference < 0 cm]. After the 1-year follow up, changes in eGFR in Group I (-6.0 ± 10.2ml/min/1.73 m^2) were lower than those in Group D (-2.7 ± 8.2ml/min/1.73 m^2), but not at a significant level ($p = 0.0599$).

Table 2 Comparison of eGFR between men with and without metabolic syndrome

	Abdominal obesity (−)	Abdominal obesity (+)	p
Number of subjects	42	78	
eGFR (ml/min/1.73 m^2)	79.1 ± 13.9	86.6 ± 13.2	**0.0042**
	Impaired glucose torelance (−)	Impaired glucose torelance (+)	
Number of subjects	96	24	
eGFR (ml/min/1.73 m^2)	82.7 ± 13.7	88.8 ± 13.8	0.0535
	Hypertension (−)	Hypertension (+)	
Number of subjects	48	72	
eGFR (ml/min/1.73 m^2)	83.2 ± 14.8	84.5 ± 13.3	0.6326
	Dyslipidemia (−)	Dyslipidemia (+)	
Number of subjects	71	49	
eGFR (ml/min/1.73 m^2)	82.3 ± 14.1	86.2 ± 13.5	0.1348
	Metabolic syndrome (−)	Metabolic syndrome (+)	
Number of subjects	84	36	
eGFR (ml/min/1.73 m^2)	82.4 ± 14.0	87.5 ± 13.2	0.0644

Mean ± SD

Table 3 Simple correlation analysis between changes in eGFR andchanges in clinical parameters with 1-year follow up

	r	p
Abdominal circumference (cm)	−0.232	**0.0106**
Systolic blood pressre (mmHg)	0.094	0.3068
Diastolic blood pressure (mmHg)	−0.009	0.9227
Triglyceride (mg/dl)	−0.055	0.5521
HDL cholesterol (mg/dl)	−0.016	0.8616
Blood sugar (mg/dl)	−0.030	0.7458

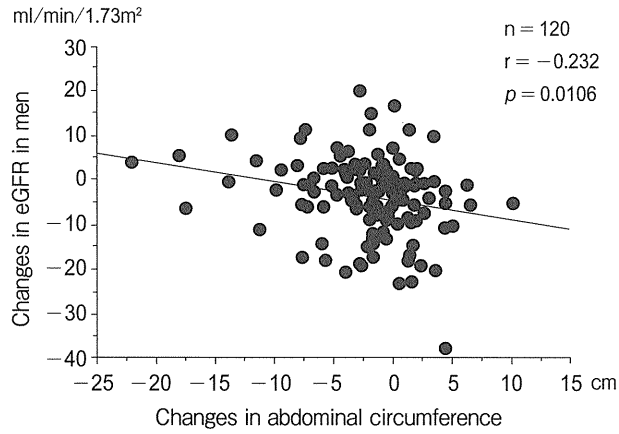

Fig. 1 Simple correlation analysis between changes in eGFR and changes in systolic blood pressure at the 1-year follow up.

Discussion

The main objective of this study was to explore the link between changes in eGFR and changes in metabolic syndrome components in Japanese men with a 1-year follow up.

Ninomiya T *et al.* [7], Tanaka *et al.* [8] and Iseki *et al.* [9] reported that metabolic syndrome, using the modified ATP III definition [10], was associated with CKD in the Japanese population. Compared with subjects with 0 or 1 components of metabolic syndrome, subjects with 2, 3, and 4 or more components had odds ratios of 1.13, 1.90, and 2.79 for CKD [7]. In this study, 36 subjects was diagnosed as having metabolic syndrome, using the Japanese criteria, at baseline, and 18 were diagnosed as having metabolic syndrome at the 1-year follow up. We have previously reported a prevalence of 30.7% for metabolic syndrome in Japanese men [11]. In this study, with lifestyle modification after the initial health check-up, metabolic components were significantly improved in men without medications at the 1-year follow-up. Although eGFR was not increased after 1 year, changes in eGFR were negatively correlated with changes in abdominal circumference. Taken together, lifestyle modification targeting reducing abdominal circumference may be a useful method for improving eGFR in Japanese men.

Abdominal obesity contributes to the development of renal injury and end-stage renal disease [12–14]. Bonnet *et al.* have reported that abdominal obesity is related to the development of elevated albuminuria in both sexes, suggesting that the measurement of abdominal circumference might improve the identification of non-diabetic individuals at risk of developing microalbuminuria [12]. In addition, a greater waist-to-hip ratio is associated with a greater risk of diminished filtration, even when corrected for BMI [13]. Yamagata *et al.* have reported that the baseline-adjusted predictor of developing CKD included age, GFR, hematuria, hypertension, diabetes, serum lipids, obesity, smoking status, and consumption of alcohol with a 10-year follow up [14]. In the present study, there were significant differences in eGFR between subjects with and without abdominal obesity at baseline. However, we revealed that, with lifestyle modification, changes in abdominal circumference were weakly correlated with changes in eGFR in men without medications. Changes in other metabolic components were not linked to changes in eGFR. Therefore, the clinical impact of adnominal circumference on eGFR was noted in Japanese men.

Potential limitations remain in our study. First, the 16,383 subjects in our study voluntarily underwent the annual health check-up; they were, therefore, probably more health-conscious than the average person. The selected 120 men underwent an annual health check-up every year with a follow-up duration of 1-year and received no medication; they were, therefore, probably even more health-conscious than most of the subjects in the database, and the small sample size may make it difficult to infer causality between eGFR and abdominal circumference. At baseline, in contrast to our previous report regarding a large sample (n = 11,711) from a cross-sectional study [4], eGFR in men with abdominal obesity was higher than that in men without abdominal obesity. eGFR was not increased with lifestyle modification after 1 year

($-3.9\,ml/min/1.73\,m^2/year$). A link has previously been found between eGFR and age, based on a large sample from a Japanese cohort, with an average decline rate of eGFR of $0.36\,ml/min/1.73\,m^2/year$ [15]. Therefore, the decline rate of eGFR in our study was higher than that previously reported. Second, we could not identify the mechanism of the linkage between eGFR and abdominal circumference. Third, most of the enrolled subjects were not diagnosed as CKD at baseline. Therefore, the results in this study may not apply to patients with CKD. Further prospective studies are needed in Japanese subjects.

In conclusion, a decrease in abdominal circumference with lifestyle modification might induce an improvement in eGFR. Therefore, lifestyle modification may be a necessary and useful measure for the prevention of CKD in Japanese men.

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References

1. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Kidney Disease Outcome Quality Initiative. Am J Kidney Dis (2002) 39: S1-S266.
2. Imai E, Horio M, Iseki K, Yamagata K, Watanabe T, Hara S, Ura N, Kiyohara Y, Hirakata H, Moriyama T, Ando Y, Nitta K, Inaguma D, Narita I, Iso H, Wakai K, Yasuda Y, Tsukamoto Y, Ito S, Makino H, Hishida A and Matsuo S: Prevalence of chronic kidney disease (CKD) in the Japanese general population predicted by the MDRD equation modified by a Japanese coefficient. Clin Exp Nephrol (2007) 11: 156-163.
3. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H and Hishida A; on behalf of the collaborators developing the Japanese equation for estimated GFR: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis (2009) 53: 982-992.
4. Miyatke N, Shikata K, Makino H and Numata T: Relationship between Estimated Glomerular Filatration Rate (eGFR) and Metabolic Syndrome in the Japanese Population. Acta Med Okayama (2010) 64: 203-208.
5. Miyatake N, Shikata K, Makino H and Numata T: Decreasing systolic blood pressure is associated with improving estimated glomerular filtration rate (eGFR) with lifestyle modification in healthy Japanese women. Acta Med Okayama (2010) 64: 339-343.
6. Definition and the diagnostic standard for metabolic syndrome-Committee to Evaluate Diagnostic Standards for Metabolic Syndrome. Nippon Naika Gakkai Zasshi (2005) 94: 794-809 (in Japanese).
7. Ninomiya T, Kiyohara Y, Kubo M, Yonemoto K, Tanizaki Y, Doi Y, Hirakata H and Iida M: Metabolic syndrome and CKD in a general Japanese population: the Hisayama Study. Am J Kidney Dis (2006) 48: 383-391.
8. Tanaka H, Shiohira Y, Uezu Y, Higa A and Iseki K: Metabolic syndrome and chronic kidney disease in Okinawa, Japan. Kidney Int (2006) 69: 369-374.
9. Iseki K, Kohagura K, Sakime A, Iseki C, Kinjo K, Ikeyama Y and Takishita S: Changes in the demographics and prevalence of chronic kidney disease in Okinawa, Japan (1993 to 2003). Hypertens Res (2007) 30: 55-62.
10. Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults: Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel Ⅲ). JAMA (2001) 285: 2486-2497.
11. Miyatake N, Kawasaki Y, Nishikawa H, Takenami S and Numata T: Prevalence of metabolic syndrome in Okayama prefecture, Japan. Intern Med (2006) 45: 107-108.
12. Bonnet F, Marre M, Halimi JM, Stengel B, Lange C, Laville M, Tichet J and Balkau B; DESIR Study Group: Waist circumference and the metabolic syndrome predict the development of elevated albuminuria in non-diabetic subjects: the DESIR Study. J Hypertens (2006) 24: 1157-1163.
13. Pinto-Sietsma SJ, Navis G, Janssen WM, de Zeeuw D, Gans RO and de Jong PE; PREVENT Study Group. A central body fat distribution is related to renal function impairment, even in lean subjects. Am J Kidney Dis (2003) 41: 733-741.
14. Yamagata K, Ishida K, Sairenchi T, Takahashi H, Ohba S, Shiigai T, Narita M and Koyama A: Risk factors for chronic kidney disease in a community-based population: a 10-year follow-up study. Kidney Int (2007) 71: 159-166.
15. Imai E, Horio M, Yamagata K, Iseki K, Hara S, Ura N, Kiyohara Y, Makino H, Hishida A and Matsuo S: Slower decline of glomerular filtration rate in the Japanese general population: a longitudinal 10-year follow-up study. Hypertens Res (2008) 31: 433-411.

Activation of Peroxisome Proliferator–Activated Receptor δ Inhibits Streptozotocin-Induced Diabetic Nephropathy Through Anti-Inflammatory Mechanisms in Mice

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OBJECTIVE—Activation of the nuclear hormone receptor peroxisome proliferator–activated receptor (PPAR)-δ has been shown to improve insulin resistance, adiposity, and plasma HDL levels. Several studies have reported that activation of PPARδ is atheroprotective; however, the role of PPARδ in renal function remains unclear. Here, we report the renoprotective effects of PPARδ activation in a model of streptozotocin-induced diabetic nephropathy.

RESEARCH DESIGN AND METHODS—Eight-week-old male C57BL/6 mice were divided into three groups: *1*) nondiabetic control mice, *2*) diabetic mice, and *3*) diabetic mice treated with the PPARδ agonist GW0742 (1 mg/kg/day). GW0742 was administered by gavage for 8 weeks after inducing diabetes.

RESULTS—GW0742 decreased urinary albumin excretion without altering blood glucose levels. Macrophage infiltration, mesangial matrix accumulation, and type IV collagen deposition were substantially attenuated by GW0742. The gene expression of inflammatory mediators in the kidney cortex, such as monocyte chemoattractant protein-1 (MCP-1) and osteopontin (OPN), was also suppressed. In vitro studies demonstrated that PPARδ activation increased the expression of anti-inflammatory corepressor B-cell lymphoma-6, which subsequently suppressed MCP-1 and OPN expression.

CONCLUSIONS—These findings uncover a previously unrecognized mechanism for the renoprotective effects of PPARδ agonists and support the concept that PPARδ agonists may offer a novel therapeutic approach for the treatment of diabetic nephropathy. ***Diabetes*** **60:960–968, 2011**

The increasing prevalence of diabetic nephropathy worldwide is a major societal issue because of the enormous expense associated with kidney replacement therapy (1). The pathogenesis of diabetic nephropathy is complex and involves multiple pathways that lead to kidney injury, including the polyol pathway (2), protein kinase C (3), advanced glycation end products (4), and transforming growth factor (TGF)-β (5). In addition, inflammation is now recognized to play an important role in the development of diabetic nephropathy (6,7). In this condition, the accumulation of macrophages and increased expression of cell adhesion molecules are observed in renal biopsy specimens from patients with diabetic nephropathy (8). We have demonstrated that inflammation in diabetic nephropathy can be ameliorated by inhibiting macrophage infiltration in intercellular adhesion molecule-1 (ICAM-1) and macrophage scavenger receptor-A (SR-A) knockout mice (9,10). Therefore, inflammation could be a major therapeutic target of diabetic nephropathy.

There is an increasing body of evidence suggesting that a subfamily of nuclear hormone receptor transcription factors, namely the peroxisome proliferator–activated receptors (PPARs) (PPARγ, PPARα, and PPARδ), may play important roles in the pathogenesis of metabolic syndrome, obesity, and diabetes (11). PPARs are also implicated in many renal pathophysiological conditions, including diabetic nephropathy and glomerulosclerosis (12). Synthetic PPARγ and PPARα agonists, such as thiazolidinediones and fibrates, improve the glycemic control in type 2 diabetic patients and lower the serum triglyceride levels in hyperlipidemic patients. In addition, we and other investigators have reported that PPARγ and PPARα agonists are also beneficial in diabetic nephropathy (13–15). Although atheroprotective effects of PPARδ agonists have been reported (16,17), there are no reports regarding the effects of PPARδ agonists on diabetic nephropathy.

The purpose of the current study was to investigate the hypothesis that activation of PPARδ prevents the development of diabetic nephropathy by inhibiting inflammatory processes, including chemokine/cytokine expression and macrophage infiltration.

RESEARCH DESIGN AND METHODS

Experimental protocol. Male C57BL/6J mice were purchased from Charles River (Yokohama, Japan). Eight-week-old mice were divided into three groups: *1*) nondiabetic control mice (control; $n = 6$), *2*) streptozotocin (STZ)–induced diabetic mice (DM; $n = 7$), and *3*) diabetic mice treated with PPARδ agonist GW0742 (DM+GW0742; $n = 7$). GW0742 mice were purchased from Sigma-Aldrich (Tokyo, Japan). Diabetes was induced by peritoneal injection of 200 mg/kg STZ (Sigma-Aldrich) in citrate buffer (pH 4.5). Blood glucose was measured by the glucose oxidase method at 3 and 7 days after STZ injection, and only mice with blood glucose concentrations >16 mmol/L were used in the study. Mice in the control group were injected with citrate buffer. The DM+GW0742 group received 1 mg/kg/day of GW0742 by gavage for 8 weeks. All mice had free access to standard diet and tap water. All procedures were performed according to the Guidelines for Animal Experiments at Okayama University Medical School, Japanese Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). Mice were killed at 8 weeks after

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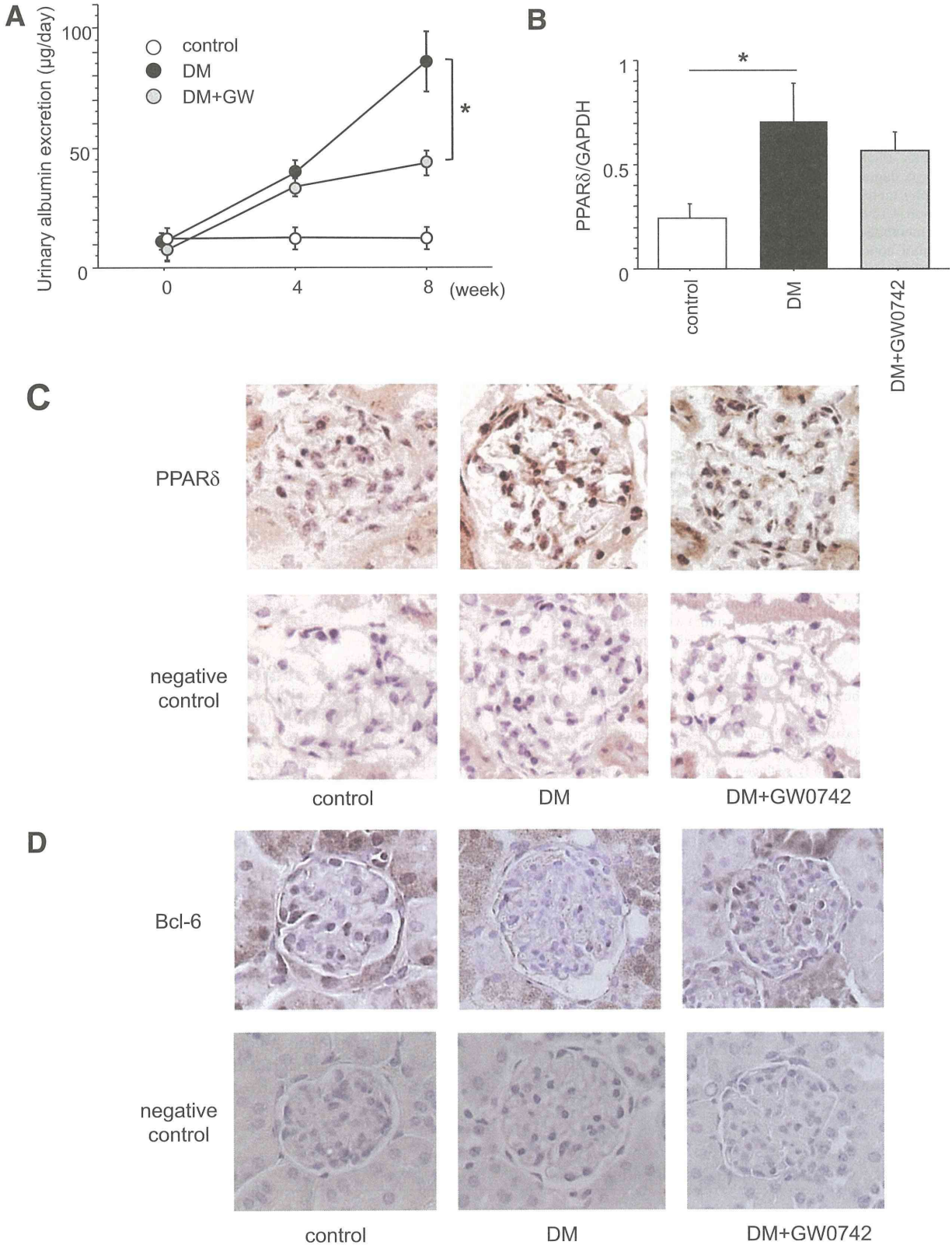

FIG. 1. Time course of changes in UAE and PPARδ mRNA and protein expression in the kidneys. *A*: The UAE increased progressively in the untreated diabetic (DM) group during the 8-week observation period after the induction of diabetes. GW0742 treatment (DM+GW0742) significantly reduced UAE at 8 weeks compared with the DM group. Control, nondiabetic control mice. Data are means ± SE. *$P < 0.01$ for DM vs. DM+GW0742. *B*: Renal PPARδ mRNA expression was significantly increased in the DM group compared with the control group. Data are means ± SE. *$P < 0.05$. *C*: Localization of renal PPARδ protein expression by immunohistochemistry. PPARδ protein expression was predominantly localized in the glomeruli of the DM and DM+GW0742 groups. Original magnification ×400. *D*: Localization of renal Bcl-6 protein expression by immunohistochemistry. Bcl-6 protein was mainly expressed in the glomeruli of the control group, but its expression was suppressed in the DM group. The expression of Bcl-6 recovered in the DM+GW0742 groups compared with the DM group. Original magnification ×400. (A high-quality digital representation of this figure is available in the online issue.)

inducing diabetes. The kidneys were removed, weighed, and fixed in 10% formalin for periodic acid–methenamine silver (PAM) staining, and parts of the remaining tissues were embedded in optimal cutting temperature compound (Sakura Finetechnical, Tokyo, Japan) and frozen immediately in acetone cooled on dry ice. Other tissues were snap-frozen in liquid nitrogen and stored at −80°C.

Metabolic data. We measured body weight, blood pressure, hemoglobin A_{1c} (HbA_{1c}), 24-h urinary albumin excretion (UAE), and creatinine clearance at 0, 4, and 8 weeks. Blood pressure was measured using the tail-cuff method (Softron, Tokyo, Japan). HbA_{1c} was measured using the high-pressure liquid chromatography method, and serum creatinine was measured using the enzymatic method. Urine was collected for 24 h, with each mouse individually housed in a metabolic cage and provided with food and water ad libitum. Urinary albumin concentration was measured as previously described (9). Creatinine was measured enzymatically, and creatinine clearance was calculated.

Light microscopy. PAM-stained sections were analyzed. To evaluate glomerular size, we examined 10 randomly selected glomeruli in the cortex per animal under high magnification (×400) at 8 weeks after induction of diabetes. The area of the glomerular tuft and the mesangial matrix index (MMI) were measured using Lumina Vision software (Mitani Corporation, Tokyo, Japan). MMI was defined as the PAM-positive area in the tuft area, calculated using the following formula: MMI = (PAM positive area)/(tuft area). The results are expressed as means ± SE (per μm^2 for tuft area; arbitrary units for MMI).

Immunoperoxidase staining. Immunoperoxidase staining was performed as previously described (9). Briefly, fresh frozen sections were cut to 4 μm thick using a cryostat. To evaluate macrophage infiltration, we applied a rat anti-mouse monocyte/macrophage (F4/80) monoclonal antibody (Abcam, Tokyo, Japan), followed by biotin-labeled goat antirat IgG antibody (Jackson Immunoresearch Laboratories, West Grove, PA). The avidin-biotin coupling reaction was performed on sections using a Vectastain Elite kit (Vector Laboratories, Burlingame, CA). We examined 10 glomeruli per animal and counted the number of F4/80-positive cells. The mean number of positive cells per glomerulus and interstitial tissue (number per mm^2) were used for the estimation. To evaluate PPARδ and Bcl-6 expression, PPARδ rabbit polyclonal antibody (Affinity BioReagents, Golden, CO) and Bcl-6 rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) were applied, followed by biotin-labeled donkey anti-rabbit IgG antibody (Jackson Immunoresearch Laboratories).

Immunofluorescent staining. Immunofluorescent staining was performed as previously described (9). To clarify the differences in mesangial matrix proteins, we used rabbit anti-type IV collagen antibody (Millipore, Temecula, CA), followed by Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen, Carlsbad, CA). Fluorescence pictures were obtained using a fluorescence microscope (BX51; Olympus, Tokyo, Japan). The type IV collagen immunofluorescence intensity was quantified as previously described (9). Briefly, using Lumina Vision software (Mitani Corporation), the intensity of expression on the images was calculated using the formula, x (density) × positive area (μm^2). The positive area of type IV collagen in each glomerulus was estimated as the ratio to the mean area of the glomerulus. Ten glomeruli per animal were evaluated.

Quantitative analysis of renal cortex gene expression. The RNA from the renal cortex was isolated 8 weeks after treatment using an RNeasy Mini Kit (Qiagen, Valencia, CA). Single-strand cDNA was synthesized from the extracted RNA using a RT-PCR kit (Perkin Elmer, Foster City, CA). To evaluate the mRNA expression of PPARδ, CD14, CD11c, monocyte chemoattractant protein (MCP)-1, chemokine CC motif receptor 2 (CCR2), TGF-β, tumor necrosis factor (TNF)-α, osteopontin (OPN), and ICAM-1 in the renal cortex, quantitative RT-PCR (qRT-PCR) was performed using StepOnePlus (Applied Biosystems, Tokyo, Japan) and FastStart SYBR Premix Ex Taq II (Takara Bio, Otsu, Japan). The primers were purchased from Takara Bio. Each sample was analyzed in triplicate and normalized for GAPDH mRNA expression.

Cell culture and treatment. RAW 264.7 murine macrophages were cultured in Dulbecco's modified Eagle's medium supplemented with 1,000 mg/L D-glucose, 10% FBS, 100 units/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L L-glutamine. For ligand treatment, cells were serum-starved by culture in 0.5% FBS for 24 h. After pretreatment with GW0742 (10 μmol/L) for 24 h, the cells were stimulated with 4,500 mg/L D-glucose (high glucose) for 24 h. Individual experiments were repeated at least three times with different lots or preparation of cells.

Quantitative analysis of gene expression in RAW macrophages. Total RNA was prepared from cells using an RNeasy Mini Kit (Qiagen) as described above. B-cell lymphoma-6 (Bcl-6), MCP-1, and OPN mRNA expression in RAW macrophages was measured using qRT-PCR, as described above.

Immunoprecipitation and Western blotting. Bcl-6 and PPARδ protein expression levels were determined by Western blotting. To examine the interactions between PPARδ and Bcl-6, the nuclear protein fraction was isolated from RAW macrophages using a Nuclear Extract Kit (Active Motif, Carlsbad, CA). The nuclear protein was immunoprecipitated with an anti-PPARδ antibody (Affinity BioReagents) for 1.5 h at 4°C. The nuclear protein–antibody complex was then incubated with magnetized Protein G Dynabeads (Invitrogen) for 45 min at room temperature. After washing the beads, the bound proteins were eluted and resolved by SDS-PAGE. Protein was transferred to nitrocellulose membranes and blocked in 20 mmol/L Tris-HCl (pH 7.6) containing 150 mmol/L NaCl, 0.1% Tween-20, and 5% (wt/vol) nonfat dried milk. The blots were then incubated with anti-PPARδ antibody (Affinity BioReagents) and anti–Bcl-6 antibody (Santa Cruz Biotechnology). The immunoblots were hybridized with anti-TATA binding protein antibody (Abcam) to monitor equivalent loading in different lanes. All experiments were repeated at least three times.

Statistical analysis. All values are means ± SE. Statistically significant differences between groups were examined using one-way ANOVA followed by Scheffé test. A P value <0.05 was considered statistically significant.

RESULTS

Metabolic data and time course of changes in UAE. The UAE progressively increased in diabetic mice during the study (Fig. 1*A*). However, GW0742 treatment significantly reduced the mean UAE (86.09 ± 12.67 μg/day) compared with the DM group at 8 weeks after inducing diabetes (40.91 ± 3.94 μg/day; $P < 0.01$). The other metabolic data are summarized in Table 1. Eight weeks after inducing diabetes, there were no significant differences in systolic blood pressure between the three groups. HbA_{1c}, kidney weight, and relative kidney weight were significantly higher in the DM group than in the control group. There was no significant difference in HbA_{1c}, kidney weight, and relative kidney weight between the DM and the DM+GW0742 groups. Body weight was lower in both the DM and the DM+GW0742 groups than in the control, but was higher in the DM+GW0742 than in the DM group. There were no significant differences in creatinine clearance or triglyceride levels between the three groups.

Renal PPARδ and Bcl-6 expression. We found PPARδ mRNA and protein expression in the kidneys. At 8 weeks,

TABLE 1
Metabolic data at 8 weeks after inducing diabetes

	Control	DM	DM+GW0742
n	6	7	7
Systolic blood pressure (mmHg)	97.8 ± 7.9	111.8 ± 4.4	110.1 ± 3.5
HbA_{1c} (%)	4.60 ± 0.04	9.75 ± 0.34*	8.81 ± 0.62*
Body weight (g)	26.75 ± 0.39	17.74 ± 1.03*	20.70 ± 0.41*†
Kidney weight (mg)	283.3 ± 3.3	325.7 ± 18.8‡	295.7 ± 6.1
Relative kidney weight (mg/g body wt)	11.82 ± 0.27	18.85 ± 1.87*	15.87 ± 0.57
Creatinine clearance (mL/min)	249.0 ± 34.8	349.5 ± 40.4	348.1 ± 44.7
Triglycerides (mg/dL)	23.6 ± 2.0	27.9 ± 7.3	21.5 ± 4.8

Data are means ± SE. *$P < 0.01$ vs. the control group. †$P < 0.01$ vs. the DM group. ‡$P < 0.05$ vs. the control group.

renal PPARδ mRNA expression was significantly greater in the DM group than in the control group (0.71 ± 0.18 vs. 0.24 ± 0.07, respectively; $P < 0.05$) (Fig. 1*B*). However, GW0742 treatment did not affect PPARδ mRNA expression in renal tissues. Renal sections immunostained with PPARδ-specific antibodies revealed that PPARδ protein expression was predominantly localized in the glomeruli of DM and DM+GW0742 groups and to a lesser extent in the glomeruli of the control group (Fig. 1*C*). By contrast, the anti-inflammatory corepressor Bcl-6 was mainly expressed in the glomeruli of the control group, and its expression was suppressed in the DM group. GW0742 treatment recovered the expression of Bcl-6 compared with the DM group (Fig. 1*D*).

MMI and expression of type IV collagens in the glomeruli. Representative glomeruli in PAM-stained sections are shown in Fig. 2*A*. Glomerular hypertrophy and mesangial matrix expansion were observed in the DM group at the end of the 8-week observation period. However, these changes were ameliorated in the DM+GW0742 group compared with DM group (MMI: 9.05 ± 0.30 vs. 12.34 ± 0.49%, respectively; $P < 0.001$) (Fig. 2*B*). A similar trend was noted for type IV collagen (Fig. 2*C*). The type IV collagen–positive area in glomeruli was larger in the DM group than in the control group. This area was markedly reduced in the DM+GW0742 group compared with the DM group (9.57 ± 0.18 vs. 12.33 ± 0.49%, respectively; $P < 0.001$) (Fig. 2*D*).

Macrophage infiltration in the kidney. The number of macrophages in the glomeruli was remarkably higher in the DM group than in the control group. Interestingly, macrophage infiltration into the glomeruli was significantly reduced in the DM+GW0742 group compared with the DM group (1.80 ± 0.08 vs. 2.69 ± 0.05, respectively; $P < 0.001$) (Fig. 3*A* and *B*). Similarly, macrophage infiltration into the interstitium was increased in the DM group but was suppressed in the DM+GW0742 group (13.79 ± 0.53 vs. 7.75 ± 0.77, respectively; $P < 0.001$) (Fig. 3*A* and *C*).

Macrophage and inflammatory gene expression in the renal cortex. qRT-PCR analyses of kidney tissue demonstrated that the expression of two macrophage marker genes, *CD14* and *CD11c*, was increased in the DM group and that GW0742 treatment markedly reduced the expression of these genes (Fig. 4). *CD14* is a marker for all macrophages, and *CD11c* is specific for the M1 subtype of macrophages. Similarly, the induction of diabetes increased the renal expression of several proinflammatory and proatherogenic genes, including *MCP-1*, *TGF-β*, *OPN*, *TNF-α*, and *ICAM-1*. Although GW0742 decreased the expression of *MCP-1*, *TGF-β*, and *OPN*, it did not affect *TNF-α* or *ICAM-1* (Fig. 4). Of note, *MCP-1*, a key chemokine involved in macrophage recruitment, plays a significant role in diabetic nephropathy because the absence of *MCP-1* significantly reduces diabetic renal injury (18,19). Similarly, *OPN* is also a critical inflammatory cytokine involved in diabetic nephropathy (20,21). Collectively, these data indicate that the PPARδ agonist GW0742 inhibits diabetes–induced macrophage recruitment and inflammatory gene expression in the kidney.

PPARδ and Bcl-6 expression in RAW macrophages. PPARδ has been demonstrated to directly affect the anti-inflammatory transcriptional repressor Bcl-6. PPARδ agonists increase free Bcl-6 in macrophages, suppressing MCP-1 transcription and decreasing macrophage infiltration (22). To investigate the regulation of renal inflammatory pathways by diabetes and PPARδ, and the roles of Bcl-6 and PPARδ in these processes, we performed in vitro

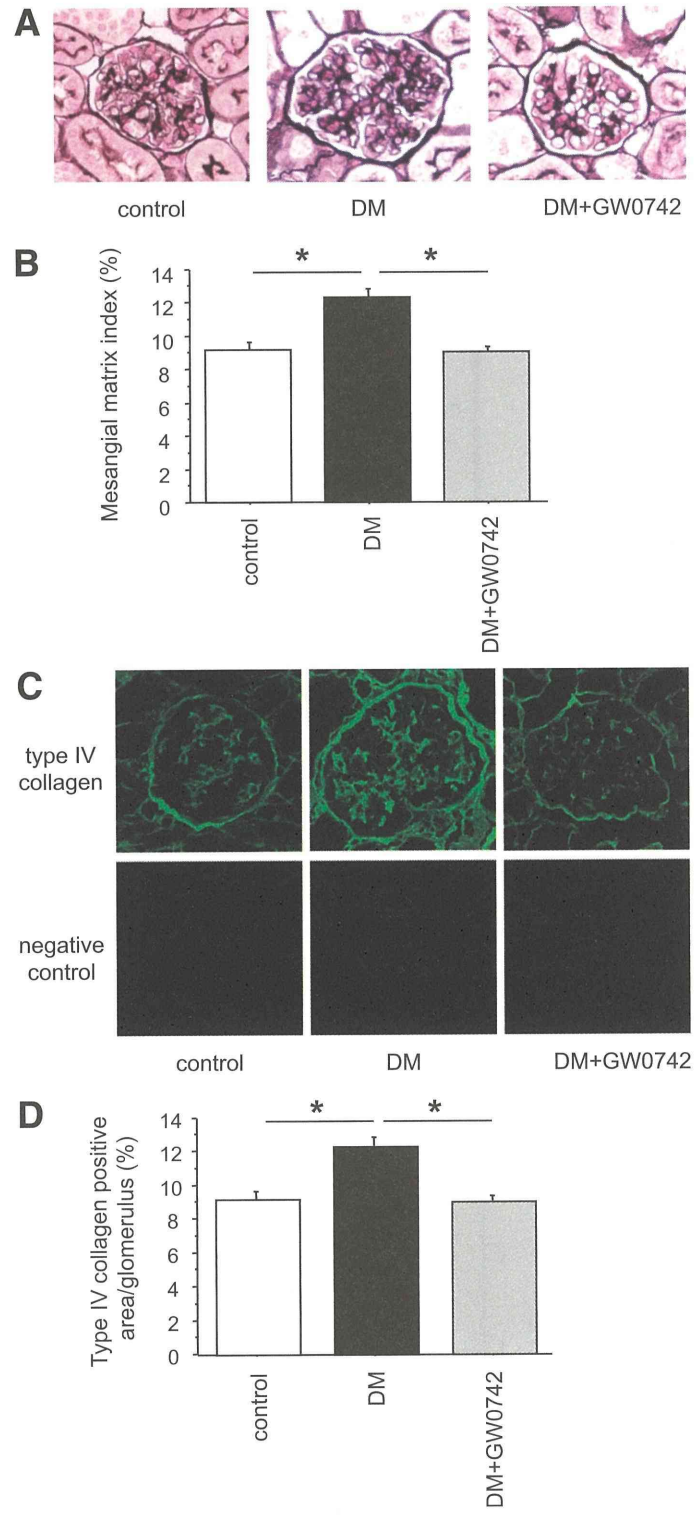

FIG. 2. PAM staining of kidney sections and the expression of type IV collagen in the kidney. *A*: Representative glomeruli from control, DM, and DM+GW0742 mice. Glomerular hypertrophy and mesangial matrix expansion were evident in the DM group. GW0742 suppressed the increase in the MMI compared with the DM group. Original magnification ×400. *B*: MMI in glomeruli. Data are means ± SE. *$P < 0.001$. *C*: Type IV collagen was significantly increased in the DM group compared with the control group and was decreased in the DM+GW0742 group compared with the DM group. Original magnification ×400. *D*: Type IV collagen–positive area in glomeruli. Data are means ± SE. *$P < 0.001$. (A high-quality digital representation of this figure is available in the online issue.)

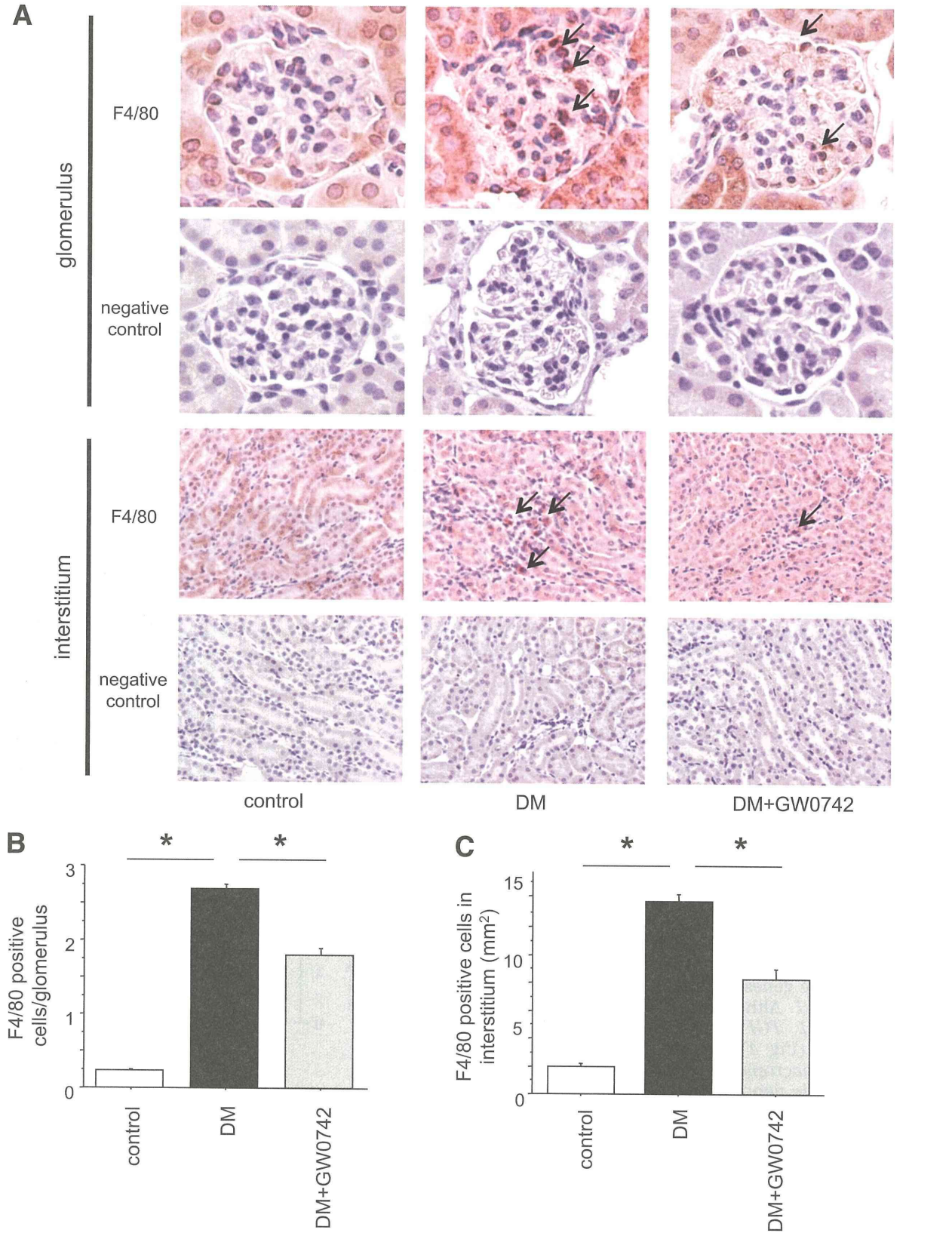

FIG. 3. Macrophage infiltration into the kidney. *A*: Macrophage (*arrows*) infiltration into glomeruli and interstitium was remarkable in the DM group and was suppressed in the DM+GW0742 group. Original magnification ×400. *B*: The number of intraglomerular macrophages. Data are means ± SE. *$P < 0.001$. *C*: The number of macrophages in interstitium. Data are means ± SE. *$P < 0.001$. (A high-quality digital representation of this figure is available in the online issue.)

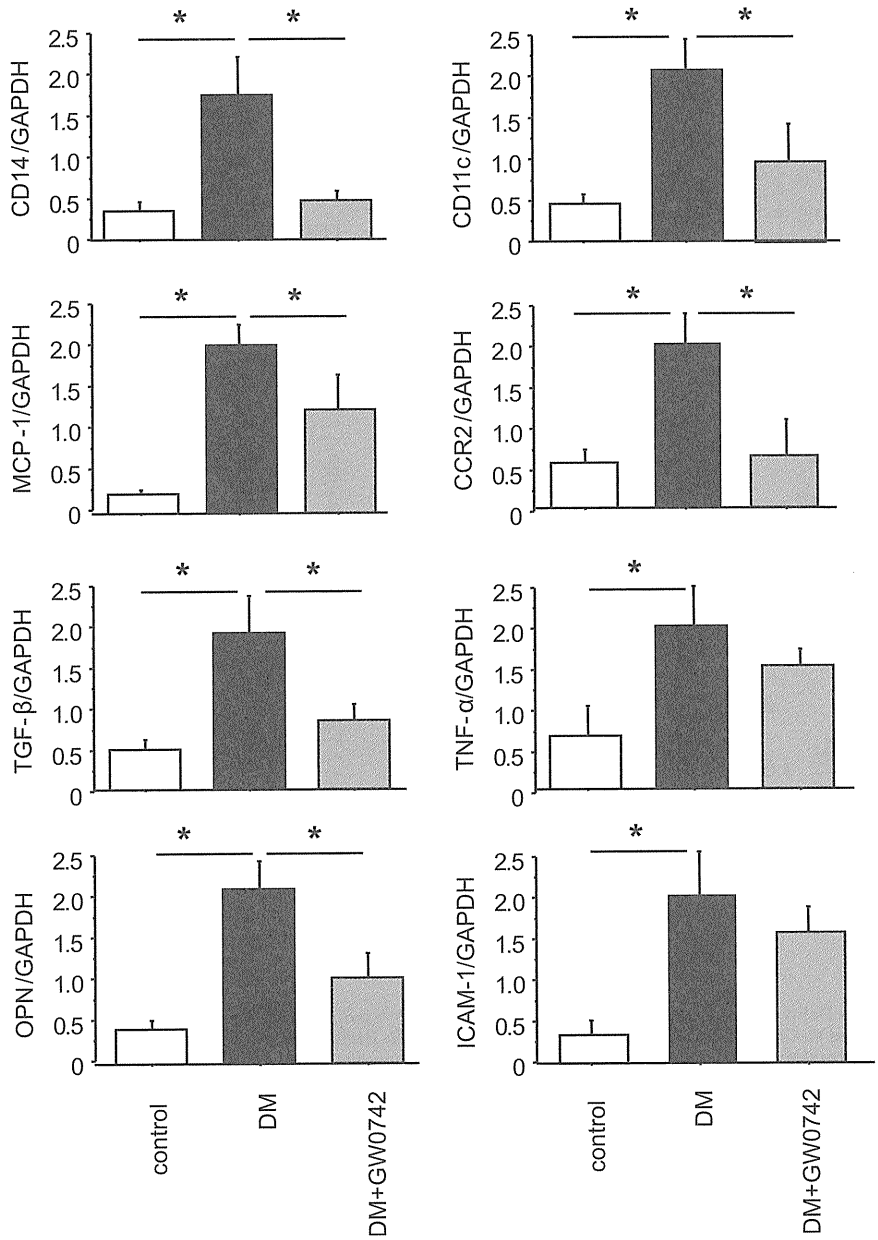

FIG. 4. PPARδ activation suppresses diabetes–induced renal inflammation and macrophage infiltration. Quantitative RT-PCR analysis of the expression of two macrophage markers (CD14 and CD11c) shows that GW0742 inhibited diabetes–induced macrophage infiltration into the kidney. Similarly, GW0742 suppressed MCP-1, CCR-2, TGF-β, and OPN mRNA levels in the kidney. mRNA levels are normalized to GAPDH. Data are means ± SE. *$P < 0.05$.

studies using RAW macrophages. qRT-PCR analyses revealed that the high-glucose medium strongly inhibited Bcl-6 expression in RAW macrophages and that GW0742 treatment significantly attenuated this inhibition in macrophages (Fig. 5*A*). We found that macrophages exposed to high glucose had an increase in nuclear PPARδ protein and that pretreatment with GW0742 completely abolished this effect (Fig. 5*B*). Western blot analyses of total and PPARδ-bound Bcl-6 in macrophage nuclear extracts revealed that high glucose tended to suppress total Bcl-6 but markedly increased PPARδ–Bcl-6 complexes and that GW0742 pretreatment decreased PPARδ–Bcl-6 binding (Fig. 5*C*). These data indicate that high glucose can increase nuclear PPARδ–Bcl-6 binding, limiting the amount of free Bcl-6 available to repress the expression of inflammatory genes normally suppressed by Bcl-6.

Inflammatory gene expression in RAW macrophages. To further investigate the role of PPARδ activation in inflammatory processes, we examined the effects of GW0742 on inflammatory gene expression in macrophages. Consistent with the changes in free Bcl-6, high glucose–stimulated MCP-1 expression, which is regulated by Bcl-6, was attenuated by GW0742 (Fig. 6*A*). The expression of OPN, a macrophage chemokine, was also increased by exposure to high glucose and suppressed by GW0742 (Fig. 6*B*).

DISCUSSION

In the current study, we demonstrated that the PPARδ agonist GW0742 ameliorated albuminuria, glomerular mesangial expansion, and type IV collagen accumulation without affecting blood glucose levels in STZ–induced