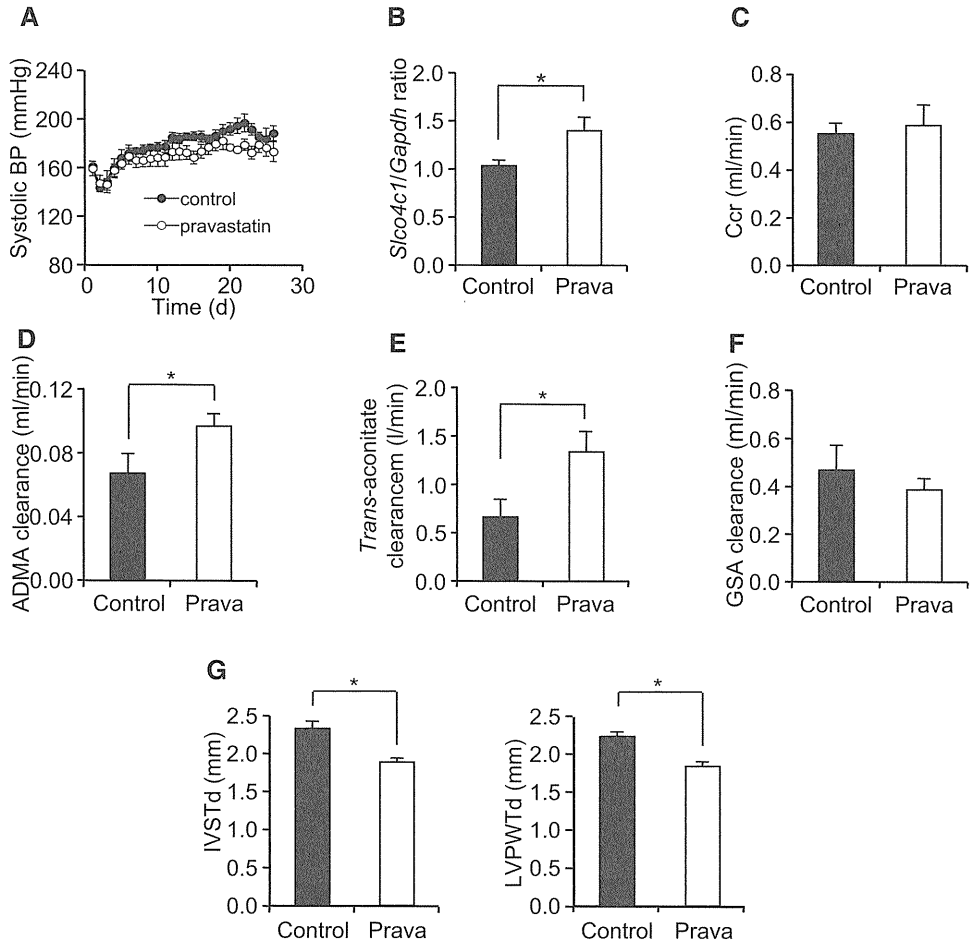

Figure 7. Effects of pravastatin *in vivo*. (A) BP in control and pravastatin-treated (0.1 mg/ml drinking water) rats after five-sixths Nx ($n = 6$ to 7 per group). (B) The mRNA expression of rat slco4c1 in the kidney after pravastatin administration ($n = 11$ per group). (C through F) Renal clearance of creatinine (C), ADMA (D), *trans*-aconitate (E), and GSA (F) 3 wk after five-sixths Nx ($n = 5$ to 7 per group). (G) Thickness of the interventricular septum (IVSTd) and left ventricular posterior wall at end-diastole (LVPWTd) before and after five-sixths Nx ($n = 6$ to 7 per group). $^*P < 0.05$.

moter.[11] The linear purified plasmid was injected into the pronuclei of fertilized oocytes of Wistar rats. Pups were analyzed for the genomic integration by Southern blotting and by PCR amplification of tail DNA using the following primers: Forward (mouse sglt2) 5′-tccccccacttctgttcccagtctatgt-3′ and reverse (human SLCO4C1) 5′-acgcgatctgcagaattagcttgggctc-3′. Reverse transcriptase–PCR was carried out using the same primers that can amplify the full length of human SLCO4C1 cDNA. Resultant TG(+) rats showed normal breeding and development with no obvious phenotypic abnormalities in body weight, water and food intake, and renal functions compared with TG(−) littermates, whose genetic background is the same as that of TG(+) rats except for expression of human SLCO4C1 (Supplemental Figure 1A). All animal experiments were approved by the Tohoku University Animal Care Committee.

Immunohistochemistry

The rabbit antiserum against 107 peptides of the *N*-terminus of human SLCO4C1 was raised and immunopurified. Western blotting and immunohistochemistry were performed as described previously,[39] and the quality was confirmed by peptide absorption (Supplemental Figure 1, B and D). The mouse mAb against CD68 was purchased from Serotec (Martinstried, Germany).

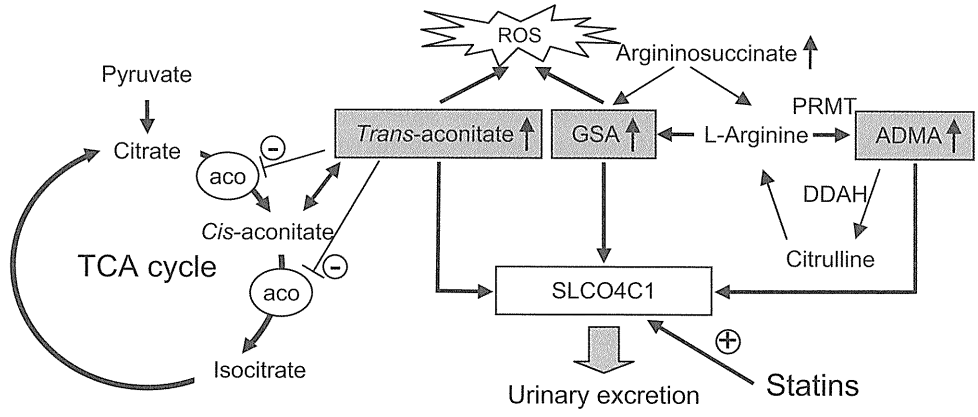

Figure 8. Uremic toxins and SLCO4C1 transporter in renal failure. ADMA is formed by protein arginine N-methyltransferase (PRMT) from arginine and degrades to citrulline by DDAH. Note that SLCO4C1 facilitates the excretion of GSA, ADMA, and *trans*-aconitate and that statins increase the expression and the function of SLCO4C1, resulting in reductions of the uremic toxins and BP. *Trans*-aconitase inhibits aconitase activity and induces reactive oxygen species (ROS). Aco, aconitase.

Nephrectomized Rat Model and BP Measurement

Five-sixths nephrectomized rats were generated as previously reported.[10] Briefly, male TG rats were intraperitoneally anesthetized with ketamine (30 mg/kg) and xylazine (2 mg/kg) and subjected to five-sixths renal ablation. At the time of surgery, rats were prepared for telemetric monitoring of BP (Data Sciences Int., St. Paul, MN).[40]

Echocardiogram

Rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) and studied with Doppler imaging by echocardiogram. The thickness of the interventricular septum and the left ventricular posterior wall at end-diastole were measured as described previously.[41]

CE-MS Method for Metabolome Analysis

A comprehensive and quantitative analysis of charged metabolites by CE-MS was performed.[13] Metabolites were first separated by CE on the basis of charge and size and then selectively detected using MS by monitoring over a large range of m/z values. Plasma and urine ADMA were measured by HPLC. Anionic and cationic compounds that were increased or decreased after Nx in both of the generated rat lines were nominated as statistically significant and are summarized in Supplemental Figure 2 (all analyzed CE-MS data are in Supplemental Tables 1 through 4). In the human plasma analysis, the protocols conformed to the ethical guidelines and approvals of both Tohoku University and Nagasaki University. Informed consent was obtained from each participant. The eGFR was calculated with the formula[42] eGFR (ml/min per 1.73 m^2) = 175 × creatinine$^{-1.154}$ × age$^{-0.203}$ × 0.742 (if female) × 0.741.

Measurement of Reactive Oxygen Species

The free radical formation within the human kidney proximal cell line HK-2 evoked by *trans*-aconitate (100 μM) was monitored by measurement of the changes in fluorescence resulting from the oxidation of dihydroethidium to ethidium as the increase of ethidium production (U/s)[43] using a 505-nm dichroic mirror with the 605/55-nm band-pass filter of an IX71 microscope (Olympus, Tokyo, Japan).

Transcriptional Assay

The human SLCO4C1 promoter DNA fragments were amplified by PCR, and the amplified fragments were inserted into the pGL3 basic luciferase expression vector (Promega, Madison, WI). The point mutation of two XREs was generated by PCR. Two micrograms of plasmid construct was transfected with 0.1 μg of *Renilla* Luciferase Reporter Vector PhRL-TK (Promega) as well as co-transfection with AhR and AhR nuclear translocator expression vector.[18] Forty-eight hours after ligand treatment, reporter assay was performed using Dual Luciferase Reporter Assay System (Promega). Incubation with activators of constitutive androstane receptor (clotrimazole and TCPOBOP), pregnane X receptor (rifampicin), and peroxisome proliferator–activated receptor α (bezafibrate, fenofibrate, clofibrate, and LTB_4) did not affect the SLCO4C1 transcription (data not shown).

ChIP Assay

ChIP assays were performed as described previously.[44] Briefly, cells either untreated or exposed to 3-MC (mouse HepaC1C7 cells) or fluvastatin (HEK293T cells) were crossed-linked with 1% formaldehyde, and protein-DNA complexes were immunoprecipitated using rabbit polyclonal antibody against AhR (BIOMOL, Plymouth, PA) or nonspecific anti-rabbit IgG. The recovered DNA was then subjected to PCR using primers that amplify regions containing the XRE elements of the human SLCO4C1 gene (forward primer 5′-AAGGGGAGCTTATGGCCA-GAGACTC-3′ and reverse primer 5′-TCGCCTCAAGGACCAACCG-GAAG-3′) or mouse cyp1a1 gene (forward primer 5′-CTATCTCTTA-AACCCCACCCCAA-3′ and reverse primer 5′-CTAAGTATGGT-GGAGGAAAGGGTG-3′). Nuclear and cytoplasmic fraction extracts were prepared and Western blotting was performed as described previously[39] using antibodies against AhR, Lamin B (Santa Cruz Biotechnology, Santa Cruz, CA), and α-tubulin (Sigma-Aldrich, St. Louis, MO).

Real-Time PCR Analysis

We performed real-time PCR analysis with probe sets from Applied Biosystems (Foster City, CA).

Statistical Analysis

The data are means ± SEM. We used an unpaired *t* test for comparisons between two groups. For multiple comparisons, we used two-way ANOVA with repeated measures in Figures 2A, 3H, and 7A and Supplemental Figure 1D and ANOVA on rank in Supplemental Figure 3, A through C. We derived *P* values for Supplemental Figure 1C using log-rank test. In Figure 4, Spearman rank correlation was calculated. $P < 0.05$ was considered to be significant.

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DISCLOSURES

None.

REFERENCES

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 351: 1296–1305, 2004
2. Kielstein JT, Zoccali C: Asymmetric dimethylarginine: A novel marker of risk and a potential target for therapy in chronic kidney disease. *Curr Opin Nephrol Hypertens* 17: 609–615, 2008
3. Marescau B, Nagels G, Possemiers I, De Broe ME, Becaus I, Billiouw JM, Lornoy W, De Deyn PP: Guanidino compounds in serum and urine of nondialyzed patients with chronic renal insufficiency. *Metabolism* 46: 1024–1031, 1997
4. Vanholder R, Van Laecke S, Glorieux G: What is new in uremic toxicity? *Pediatr Nephrol* 23: 1211–1221, 2008
5. Torremans A, Marescau B, Kranzlin B, Gretz N, Billiouw JM, Vanholder R, De Smet R, Bouwman K, Brouns R, De Deyn PP: Biochemical validation of a rat model for polycystic kidney disease: Comparison of guanidino compound profile with the human condition. *Kidney Int* 69: 2003–2012, 2006
6. Zoccali C, Mallamaci F, Maas R, Benedetto FA, Tripepi G, Malatino LS,

Cataliotti A, Bellanuova I, Boger R: Left ventricular hypertrophy, cardiac remodeling and asymmetric dimethylarginine (ADMA) in hemodialysis patients. *Kidney Int* 62: 339–345, 2002
7. Fliser D, Kronenberg F, Kielstein JT, Morath C, Bode-Boger SM, Haller H, Ritz E: Asymmetric dimethylarginine and progression of chronic kidney disease: The Mild to Moderate Kidney Disease Study. *J Am Soc Nephrol* 16: 2456–2461, 2005
8. Sanaka T, Akizawa T, Koide K, Koshikawa S: Protective effect of an oral adsorbent on renal function in chronic renal failure: Determinants of its efficacy in diabetic nephropathy. *Ther Apher Dial* 8: 232–240, 2004
9. Owada A, Nakao M, Koike J, Ujiie K, Tomita K, Shiigai T: Effects of oral adsorbent AST-120 on the progression of chronic renal failure: A randomized controlled study. *Kidney Int Suppl* 63: S188–S190, 1997
10. Mikkaichi T, Suzuki T, Onogawa T, Tanemoto M, Mizutamari H, Okada M, Chaki T, Masuda S, Tokui T, Eto N, Abe M, Satoh F, Unno M, Hishinuma T, Inui K, Ito S, Goto J, Abe T: Isolation and characterization of a digoxin transporter and its rat homologue expressed in the kidney. *Proc Natl Acad Sci U S A* 101: 3569–3574, 2004
11. Rubera I, Poujeol C, Bertin G, Hasseine L, Counillon L, Poujeol P, Tauc M: Specific Cre/Lox recombination in the mouse proximal tubule. *J Am Soc Nephrol* 15: 2050–2056, 2004
12. Silverstein DM: Inflammation in chronic kidney disease: Role in the progression of renal and cardiovascular disease. *Pediatr Nephrol* 24: 1445–1452, 2008
13. Soga T, Ohashi Y, Ueno Y, Naraoka H, Tomita M, Nishioka T: Quantitative metabolome analysis using capillary electrophoresis mass spectrometry. *J Proteome Res* 2: 488–494, 2003
14. Baylis C: Arginine, arginine analogs and nitric oxide production in chronic kidney disease. *Nat Clin Pract Nephrol* 2: 209–220, 2006
15. Levillain O, Marescau B, Possemiers I, Al Banchaabouchi M, De Deyn PP: Influence of 72% injury in one kidney on several organs involved in guanidino compound metabolism: A time course study. *Pflugers Arch* 442: 558–569, 2001
16. Saffran M, Prado JL: Inhibition of aconitase by trans-aconitate. *J Biol Chem* 180: 1301–1309, 1949
17. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubina P, Lahera V, Luno J: Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int Suppl* S4–S9, 2008
18. Fujii-Kuriyama Y, Mimura J: Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. *Biochem Biophys Res Commun* 338: 311–317, 2005
19. Wu L, Whitlock JP Jr: Mechanism of dioxin action: Receptor-enhancer interactions in intact cells. *Nucleic Acids Res* 21: 119–125, 1993
20. Nioi P, Hayes JD: Contribution of NAD(P)H:quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. *Mutat Res* 555: 149–171, 2004
21. Denison MS, Nagy SR: Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol* 43: 309–334, 2003
22. Agarwal R: Effects of statins on renal function. *Mayo Clin Proc* 82: 1381–1390, 2007
23. Kawano H, Yano K: Pravastatin decreases blood pressure in hypertensive and hypercholesterolemic patients receiving antihypertensive treatment. *Circ J* 70: 1116–1121, 2006
24. Golomb BA, Dimsdale JE, White HL, Ritchie JB, Criqui MH: Reduction in blood pressure with statins: Results from the UCSD Statin Study, a randomized trial. *Arch Intern Med* 168: 721–727, 2008
25. Yin QF, Xiong Y: Pravastatin restores DDAH activity and endothelium-dependent relaxation of rat aorta after exposure to glycated protein. *J Cardiovasc Pharmacol* 45: 525–532, 2005
26. Cohen BD: Methyl group deficiency and guanidino production in uremia. *Mol Cell Biochem* 244: 31–36, 2003
27. Watanabe K, Katsuhara M, Nakao H, Sato M: Detection and molecular analysis of plant- and insect-associated bacteria harboring aconitate isomerase involved in biosynthesis of trans-aconitic acid as antifeedant in brown planthoppers. *Curr Microbiol* 35: 97–102, 1997
28. Tong WH, Rouault TA: Metabolic regulation of citrate and iron by aconitases: Role of iron-sulfur cluster biogenesis. *Biometals* 20: 549–564, 2007
29. Campese VM, Park J: HMG-CoA reductase inhibitors and the kidney. *Kidney Int* 71: 1215–1222, 2007
30. Oguz A, Uzunlulu M: Short term fluvastatin treatment lowers serum asymmetric dimethylarginine levels in patients with metabolic syndrome. *Int Heart J* 49: 303–311, 2008
31. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ: Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem* 279: 23847–23850, 2004
32. Hu W, Sorrentino C, Denison MS, Kolaja K, Fielden MR: Induction of cyp1a1 is a nonspecific biomarker of aryl hydrocarbon receptor activation: Results of large scale screening of pharmaceuticals and toxicants *in vivo* and *in vitro*. *Mol Pharmacol* 71: 1475–1486, 2007
33. Bain MA, Faull R, Fornasini G, Milne RW, Evans AM: Accumulation of trimethylamine and trimethylamine-N-oxide in end-stage renal disease patients undergoing haemodialysis. *Nephrol Dial Transplant* 21: 1300–1304, 2006
34. Ceballos I, Chauveau P, Guerin V, Bardet J, Parvy P, Kamoun P, Jungers P: Early alterations of plasma free amino acids in chronic renal failure. *Clin Chim Acta* 188: 101–108, 1990
35. McGregor DO, Dellow WJ, Lever M, George PM, Robson RA, Chambers ST: Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations. *Kidney Int* 59: 2267–2272, 2001
36. Kand'ar R, Zakova P: Allantoin as a marker of oxidative stress in human erythrocytes. *Clin Chem Lab Med* 46: 1270–1274, 2008
37. Reddy V, Bhandari S, Seymour AM: Myocardial function, energy provision, and carnitine deficiency in experimental uremia. *J Am Soc Nephrol* 18: 84–92, 2007
38. Swendseid ME, Wang M, Vyhmeister I, Chan W, Siassi F, Tam CF, Kopple JD: Amino acid metabolism in the chronically uremic rat. *Clin Nephrol* 3: 240–246, 1975
39. Abe T, Unno M, Onogawa T, Tokui T, Kondo TN, Nakagomi R, Adachi H, Fujiwara K, Okabe M, Suzuki T, Nunoki K, Sato E, Kakyo M, Nishio T, Sugita J, Asano N, Tanemoto M, Seki M, Date F, Ono K, Kondo Y, Shiiba K, Suzuki M, Ohtani H, Shimosegawa T, Iinuma K, Nagura H, Ito S, Matsuno S: LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. *Gastroenterology* 120: 1689–1699, 2001
40. Watanabe T, Tan N, Saiki Y, Makisumi T, Nakamura S: Possible involvement of glucocorticoids in the modulation of interleukin-1-induced cardiovascular responses in rats. *J Physiol* 491: 231–239, 1996
41. Fukui S, Fukumoto Y, Suzuki J, Saji K, Nawata J, Tawara S, Shinozaki T, Kagaya Y, Shimokawa H: Long-term inhibition of Rho-kinase ameliorates diastolic heart failure in hypertensive rats. *J Cardiovasc Pharmacol* 51: 317–326, 2008
42. Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Tsukamoto Y, Ito S, Makino H, Hishida A, Matsuo S: Modification of the Modification of Diet in Renal Disease (MDRD) Study equation for Japan. *Am J Kidney Dis* 50: 927–937, 2007
43. Bindokas VP, Jordan J, Lee CC, Miller RJ: Superoxide production in rat hippocampal neurons: Selective imaging with hydroethidine. *J Neurosci* 16: 1324–1336, 1996
44. Wang S, Hankinson O: Functional involvement of the Brahma/SWI2-related gene 1 protein in cytochrome P4501A1 transcription mediated by the aryl hydrocarbon receptor complex. *J Biol Chem* 277: 11821–11827, 2002

See related editorial, "Harnessing Transporters to Clear Uremic Toxins," on pages 2483–2484.

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Relationship between Estimated Glomerular Filtration Rate (eGFR) and Metabolic Syndrome in Japanese

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Relationship between Estimated Glomerular Filtration Rate (eGFR) and Metabolic Syndrome in Japanese

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Abstract

We investigated the link between renal function as evaluated by estimated glomerular filtration rate (eGFR) and metabolic syndrome in Japanese. A total of 11,711 Japanese subjects, aged 20-79 years, were recruited in a cross-sectional clinical investigation. From this group, we further investigated the data on 1,576 subjects. eGFR was calculated using serum creatinine (Cr), age and sex. The diagnosis of metabolic syndrome was based on the Japanese criteria. In the first analysis, 288 men (7.8%) and 498 women (6.2%) were diagnosed with reduced eGFR (<60ml/min). eGFR was not correlated with anthropometric, body composition parameters in either sex. In the second analysis, in subjects without medications, 132 men (20.8%) and 15 women (1.6%) were diagnosed with metabolic syndrome. eGFR was lower in men with abdominal obesity and in women with hypertension was than in those without. Among Japanese not taking medications, lower eGFR may be a characteristic of men with abdominal obesity and of women with hypertension.

KEYWORDS: metabolic syndrome, estimated glomerular filtration rate (eGFR), abdominal circumference

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Original Article

Relationship between Estimated Glomerular Filtration Rate (eGFR) and Metabolic Syndrome in Japanese

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We investigated the link between renal function as evaluated by estimated glomerular filtration rate (eGFR) and metabolic syndrome in Japanese. A total of 11,711 Japanese subjects, aged 20-79 years, were recruited in a cross-sectional clinical investigation. From this group, we further investigated the data on 1,576 subjects. eGFR was calculated using serum creatinine (Cr), age and sex. The diagnosis of metabolic syndrome was based on the Japanese criteria. In the first analysis, 288 men (7.8%) and 498 women (6.2%) were diagnosed with reduced eGFR (< 60 ml/min). eGFR was not correlated with anthropometric, body composition parameters in either sex. In the second analysis, in subjects without medications, 132 men (20.8%) and 15 women (1.6%) were diagnosed with metabolic syndrome. eGFR was lower in men with abdominal obesity and in women with hypertension was than in those without. Among Japanese not taking medications, lower eGFR may be a characteristic of men with abdominal obesity and of women with hypertension.

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

Chronic kidney disease (CKD) has become an important public health challenge in Japan and is a major risk factor for end-stage renal disease, cardiovascular disease and premature death [1, 2]. Identifying and treating risk factors for early chronic kidney disease may be the best approach to preventing and delaying adverse outcomes [1]. In Japan, clinical practice guidelines established by the Japanese Society of Nephrology estimate that 18.7% of adults have CKD, which is defined as kidney damage or a glomerular filtration rate (GFR) <60 ml/min/1.73m^2 for at least 3 months regardless of cause [3], and that 4.1% have moderate or severe CKD [4].

Metabolic syndrome is characterized by abdominal obesity, high blood pressure, dyslipidemia and impaired glucose tolerance [5]. In Japan, according to the criteria for this syndrome as defined in April 2005, 30.7% of men and 3.6% of women have metabolic syndrome [6, 7]. In some studies, CKD is closely related to body composition parameters and metabolic syndrome [8-18]. However, the link between renal function evaluated by estimated GFR (eGFR) and metabolic syndrome components using the Japanese criteria remains to be investigated.

In this study, we investigated renal function evaluated by eGFR in Japanese and evaluated the clinical impact of metabolic syndrome on eGFR in subjects not taking medications.

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Subjects and Methods

Subjects. In the first analysis, we used all data on 11,711 Japanese (3,674 men and 8,037 women) aged 20–79 years in a cross-sectional study. All subjects met the following criteria: (1) they had been wanting to change their lifestyle *i.e.*, diet and exercise habits, and had received an annual health checkup from June 1997 to May 2007 at Okayama Southern Institute of Health; (2) their creatinine (Cr) and anthropometric measurements had been taken as part of their annual health checkups; and (3) they provided informed consent (Table 1).

In the second analysis, among the 11,711 subjects, we further examined the data on 1,576 subjects (636 men and 940 women) who undertook fasting blood examination and blood pressure measurements and who were currently taking no medications; we also examined the Cr and anthropometric measurements of these second-analysis subjects (Table 2). In addition, medical staff subjectively evaluated these subjects' lifestyles, and encouraged subjects with fasting plasma glucose $\geqq 126$ mg/dl to begin taking medication.

The study was approved by the Ethics Committee

Table 1 Clinical profiles of subjects in the first analysis

	Men (n = 3,674)			Women (n = 8,037)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age	43.8 ± 14.2	20	79	42.9 ± 14.1	20	79
Height (cm)	168.9 ± 6.2	143.7	187.6	156.2 ± 5.7	134.9	179.3
Body weight (kg)	70.3 ± 11.7	39.1	175.7	55.1 ± 9.0	32.1	116.9
BMI (kg/m^2)	24.6 ± 3.7	13.6	61.5	22.6 ± 3.6	12.9	48.7
Body fat percentage (%)	24.3 ± 6.7	1.2	47.9	30.7 ± 7.0	3.9	56.2
Abdominal circumference (cm)	84.3 ± 10.2	58.0	157.0	72.3 ± 9.7	43.3	123.6
Hip circumference (cm)	94.2 ± 6.3	71.0	145.5	91.0 ± 6.0	58.5	132.0
Cr (mg/dl)	0.83 ± 0.15	0.39	2.57	0.61 ± 0.21	0.20	8.63
eGFR (ml/min/1.73m^2)	84.8 ± 18.7	20.2	191.3	90.6 ± 22.7	4.3	260.0

BMI: body mass index
Cr: creatinine
eGFR: estimated glomerular filtration rate

Table 2 Clinical profiles of subjects in the second analysis

	Men (n = 636)			Women (n = 940)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age	43.8 ± 11.2	20	78	45.7 ± 11.6	20	76
Height (cm)	169.1 ± 6.0	146.9	187.6	156.7 ± 5.5	139.3	176.3
Body weight (kg)	71.5 ± 11.2	40.1	121.7	56.0 ± 8.9	37.1	105.3
BMI (kg/m^2)	25 ± 3.5	16.4	43.3	22.8 ± 3.5	15.7	41.3
Body fat percentage (%)	24.4 ± 6.3	2.2	41.3	31.1 ± 6.6	10.6	50.1
Abdominal circumference (cm)	84.7 ± 9.5	58.8	123.0	72.5 ± 9.0	55.5	115.6
Hip circumference (cm)	94.9 ± 5.8	79.1	121.0	91.3 ± 6.1	60.0	122.0
Cr (mg/dl)	0.83 ± 0.14	0.50	1.85	0.62 ± 0.12	0.36	1.10
eGFR (ml/min/1.73m^2)	84.0 ± 16.8	36.0	146.5	84.5 ± 18.7	38.3	166.8
Systolic blood pressure (mmHg)	129.6 ± 15.7	90.0	205.0	121.3 ± 16.4	88.0	193.0
Diastolic blood pressure (mmHg)	81.2 ± 11.1	33.0	131.0	75.2 ± 10.2	44.0	120.0
Triglyceride (mg/dl)	142.5 ± 116.8	29.0	1,683.0	93.6 ± 14.7	70.0	331.0
HDL cholesterol (mg/dl)	55.4 ± 14.6	18.0	120.0	67.2 ± 16.4	28.0	151.0
Blood sugar (mg/dl)	100.6 ± 16.8	63.0	218.0	93.6 ± 14.7	70.0	331.0

BMI: body mass index
Cr: creatinine
eGFR: estimated glomerular filtration rate

of Okayama Health Foundation.

Anthropometric and body composition measurements. The anthropometric parameters were evaluated by using the following respective parameters such as height, body weight, body mass index (BMI), abdominal circumference, and hip circumference. BMI was calculated by weight/[height]^2 (kg/m^2). The abdominal circumference was measured at the umbilical level and the hip was measured at the widest circumference over the trochanter in standing subjects after normal expiration [19]. Body fat percentage was measured by an air displacement plethysmograph called the BOD POD Body Composition System (Life Measurement Instruments, Concord, CA, USA) [20, 21].

Blood pressure measurements. Each participant's blood pressure was measured after resting at least 15 min in the sitting position.

Blood sampling and assays. The level of Cr was measured with an automated biochemical analyzer (model 7700; HITACHI, Tokyo, Japan) and Accuras Auto CRE (Shino-Test Corporation, Tokyo, Japan). High-density lipoprotein (HDL) cholesterol [22], triglycerides (L Type Wako Triglyceride·H, Wako Chemical, Osaka, Japan) and plasma glucose (hexokinase method) were also measured at the Okayama Southern Institute of Health, Okayama Health Foundation. The accuracy of the measurements was maintained during the study period. eGFR was calculated using the following equation: eGFR (ml/min/1.73 m^2) $=194 \times Cr^{-1.094} \times Age^{-0.287}$ (for men) and eGFR (ml/min/1.73 m^2) $= 194 \times Cr^{-1.094} \times Age^{-0.287} \times 0.739$ (for women) [23]. Reduced eGFR was defined as an eGFR <60 ml/min/1.73 m^2.

Definition of metabolic syndrome. The syndrome was defined [6], among men with an abdominal circumference in excess of 85 cm and women with an abdominal circumference in excess of 90 cm [24], as having 2 or more of the following components: 1) dyslipidemia: triglyceride $\geqq$ 150 mg/dl and/or HDL cholesterol $<$ 40 mg/dl; 2) hypertension: blood pressure $\geqq$ 130/85 mmHg; 3) Impaired glucose tolerance: fasting plasma glucose $\geqq$ 110 mg/dl.

Statistical analysis. Data are expressed as means $\pm$ standard deviation (SD) values. A comparison of parameters between the 2 groups was made using the unpaired t-test and covariance analysis. Simple correlation analysis was performed as well to test for the significance of the linear relationship among continuous variables: $p < 0.05$ was considered statistically significant. Statistical analysis was performed with StatView 5.0 (SAS Institute Inc., Cary, NC, USA).

Results

In the first analysis, the mean eGFR was 84.8 $\pm$ 18.7 ml/min/1.73 m^2 in men and 90.6 $\pm$ 22.7 ml/min/1.73 m^2 in women (Table 1). A diagnosis of reduced eGFR was made for 288 men (7.8%) and 498 women (6.2%). eGFR was not clearly correlated with anthropometric, body composition parameters in either sex (Table 3). eGFR in men with abdominal obesity (81.8 $\pm$ 17.8 ml/min/1.73 m^2) was lower than that in men without abdominal obesity (87.4 $\pm$ 19.1 ml/min/1.73 m^2), but the difference was not significant after adjusting for age ($p = 0.0675$). eGFR in women with abdominal obesity (83.8 $\pm$ 22.2 ml/min/1.73 m^2) was similar to that in women without abdominal obesity after adjusting for age (91.0 $\pm$ 22.6 ml/min/1.73 m^2) ($p = 0.8039$).

In the second analysis, we clarified the prevalence of metabolic syndrome among subjects who were not taking without medications (Table 4). Among the 1,576 Japanese subjects, 306 men (48.1%) had an abdominal circumference in excess of 85 cm and 48 women (5.1%) had an abdominal circumference

Table 3 Relationship between eGFR and anthropometric, body composition parameters

	Men		Women	
	r	p	r	p
Body weight (kg)	−0.017	0.2929	−0.110	<0.0001
BMI (kg/m^2)	−0.086	<0.0001	−0.174	<0.0001
Body fat percentage (%)	−0.146	<0.0001	−0.205	<0.0001
Abdominal circumference	−0.142	<0.0001	−0.233	<0.0001
Hip circumference (cm)	−0.006	0.7210	−0.060	<0.0001

Table 4 Comparison of eGFR between subjects with and without metabolic syndrome

Men	Abdominal obesity (+)	Abdominal obesity (−)	p	p (Afrer adjusting for age)
Number of subjects	306	330		
eGFR (ml/min/1.73m²)	83.8 ± 14.9	84.1 ± 18.4	0.7865	0.0055
	Impaired glucose torelance (+)	Impaired glucose torelance (−)		
Number of subjects	104	532		
eGFR (ml/min/1.73m²)	86.9 ± 16.4	83.4 ± 16.8	0.0479	0.0880
	Hypertension (+)	Hypertension (−)		
Number of subjects	347	289		
eGFR (ml/min/1.73m²)	82.7 ± 16.0	85.5 ± 17.6	0.0338	0.1106
	Dyslipidemia (+)	Dyslipidemia (−)		
Number of subjects	223	413		
eGFR (ml/min/1.73m²)	83.1 ± 16.6	84.4 ± 16.9	0.3501	0.6986
	Metabolic syndrome (+)	Metabolic syndrome (−)		
Number of subjects	132	504		
eGFR (ml/min/1.73m²)	83.6 ± 15.7	84.1 ± 17.1	0.7632	0.0830
Women	Abdominal obesity (+)	Abdominal obesity (−)		
Number of subjects	48	892		
eGFR (ml/min/1.73m²)	84.8 ± 16.7	84.5 ± 18.8	0.9179	0.2654
	Impaired glucose torelance (+)	Impaired glucose torelance (−)		
Number of subjects	50	890		
eGFR (ml/min/1.73m²)	86.0 ± 18.1	84.4 ± 18.8	0.5651	0.8745
	Hypertension (+)	Hypertension (−)		
Number of subjects	300	640		
eGFR (ml/min/1.73m²)	80.6 ± 17.0	86.3 ± 19.3	<0.0001	0.0222
	Dyslipidemia (+)	Dyslipidemia (−)		
Number of subjects	108	832		
eGFR (ml/min/1.73m²)	80.6 ± 20.0	85.0 ± 18.5	0.0223	0.2757
	Metabolic syndrome (+)	Metabolic syndrome (−)		
Number of subjects	15	925		
eGFR (ml/min/1.73m²)	81.5 ± 17.0	84.6 ± 18.8	0.5297	0.1077

Mean ± SD

exceeding 90 cm. In addition, 132 men (20.8%) and only 15 women (1.6%) were diagnosed with the syndrome.

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 4). To avoid the influence of age, we used age as a covariate and compared eGFR between Japanese with and those without metabolic syndrome components using covariance analysis. eGFR in men with abdominal obesity and in women with hypertension was significantly lower than in subjects without these components of metabolic syndrome, even after adjusting for age. However, there were no significant differences in 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, even after adjusting for age.

Discussion

Obesity is a significant risk factor for developing CKD and proteinuria [8–11]. Fox *et al.* reported that the odds ratio (OR) for developing new-onset kidney disease, defined as a GFR < 59.3 ml/min/1.73 m² in

women and 64.3 ml/min/m^2 in men, was 1.23, representing a 23% increase in BMI within 10 -years [8]. In Japan, it was also reported that BMI above 25 kg/m^2 was linked to proteinuria [9]. Bonnet *et al.* reported that abdominal obesity was 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 [10]. In addition, a greater waist-to-hip ratio was associated with a greater risk for diminished filtration, even when corrected for BMI [11]. In this study, the relationships between eGFR and anthropometric, body composition parameters were not clearly revealed in the first analysis. However, after adjusting for age by using covariance analysis, eGFR in men with abdominal obesity tended to be lower than that in men without abdominal obesity in the first and second analyses. Therefore, we could not accurately prove a link between eGFR and anthropometric, body composition parameters, unlike the case in previous studies.

This study is the first to reveal a relationship between eGFR and metabolic syndrome, defined by the new Japanese criteria of metabolic sundrome. Metabolic syndrome has important clinical and public health implications in Japan because it is a common disorder in that country [7]. Previous studies have documented that metabolic syndrome is an important risk factor for diabetes, coronary heart disease and stroke [25-27]. The present study shows new and important information about the relationship between eGFR and metabolic syndrome in a large sample of Japanese.

Subjects with metabolic syndrome, using the modified Adult Treatment Panel (ATP) III definition [28], showed higher urinary albumin excretion and left ventricular mass index, increased intima-media thickness, and a higher prevalence of microalbuminuria [12]. Compared with subjects with 0 or 1 component of the metabolic syndrome, subjects with 2, 3, 4, or 5 components of the syndrome had multivariate-adjusted odds ratios of 2.21, 3.38, 4.23, and 5.85 for CKD [13]. Using the Japanese criteria, we previously reported that the prevalence of proteinuria in subjects with metabolic syndrome was significantly higher than that in subjects without the syndrome [14]. Tanaka *et al.* [15], Ninomiya T *et al.* [16] and Iseki *et al.* [17] reported that metabolic syndrome, using the modified ATP III definition, was associated with CKD in Japanese. Although Tsuda *et al.* [18] revealed that the level of microalbuminuria in subjects with metabolic syndrome according to the Japanese criteria was significantly higher than that in subjects without the syndrome, the link between eGFR and metabolic syndrome using the Japanese criteria has not been investigated until now. In this study, although we evaluated eGFR in subjects without medications, the clinical impact of abdominal obesity in men and hypertension in women was noted in the second analysis. However, eGFR in subjects with metabolic syndrome was similar to that in subjects without the syndrome in either sex. eGFR was higher in subjects with impaired glucose tolerance than in those without, but not significantly. Glomerular hyperfiltration exists among Japanese type 2 diabetic patients with no evidence of overt proteinuria or hypertension [29]. In addition, according to the analysis of subjects without medications, the link between eGFR and metabolic syndrome and its components may be attenuated. Therefore, a significant difference in eGFR between subjects with and without metabolic syndrome might not be noted.

Potential limitations remain in this study. First, our study was a cross sectional and not a longitudinal study. Second, the 11,711 subjects, all of whom wanted to change their lifestyle, underwent measurements for this study: they were therefore more health-conscious than the average person. The selected 1,576 subjects underwent fasting blood examination and blood pressure measurements and were taking no medications; they were therefore more health-conscious than most of the subjects in the first analysis. Although some subjects were within the range of fasting plasma glucose levels at wthich medications are recommended, the prevalence of metabolic syndrome in this study was lower than in our previous report [7]. This was especially true in women, only 15 of whom were diagnosed as having metabolic syndrome. The small sample size in women with metabolic syndrome might make it difficult to compare eGFR between women with the syndrome and those without. Third, we could not accurately prove the mechanism between lower eGFR and metabolic syndrome components. Further prospective studies are needed in Japanese subjects using the new Japanese criteria.

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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. Foley RN, Parfrey PS and Sarnak MJ: Clinical epidemiology of cardiovascular disease in choronic renal disease. Am J Kidney Dis (1998) 32: S112-S119.
3. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N and Eknoyan G: Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int (2005) 67: 2089-2100.
4. 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.
5. Reaven GM: Banting Lecture 1988. Role of insulin resistance in human disease. Diabetes (1988) 37: 1595-1607.
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. 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.
8. Fox CS, Larson MG, Leip EP, Culleton B, Wilson PW and Levy D: Predictors of new-onset kidney disease in a community-based population. JAMA (2004) 291: 844-850.
9. 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.
10. 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.
11. 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.
12. Leoncini G, Ratto E, Viazzi F, Vaccaro V, Parodi D, Parodi A, Falqui V, TomolilloC, Deferrari G and Pontremoli R: Metabolic syndrome is associated with early signs of organ damage in nondiabetic, hypertensive patients. J Intern Med (2005) 257: 454-460.
13. Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK and He J: The metabolic syndrome and chronic kidney disease in U.S. adults. Ann Intern Med (2004) 140: 167-174.
14. Miyatake N, Wada J, Kawasaki Y, Matsumoto S, Makino H and Numata H: Relationship between metabolic syndrome and proteinuria in the Japanese population. Intern Med (2006) 45: 599-603.
15. 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.
16. 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.
17. 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.
18. Tsuda S, Nakano S, Konishi K and Koya D: A lack of increase in high molecular weight-adiponectin in macroalbuminuric subjects with metabolic syndrome may exert renal and atherosclerotic risks. Diabetes Res Clin Pract (2008) 79: 503-509.
19. Miyatake N, Takahashi K, Wada J, Nishikawa H, Morishita A, Suzuki H, Kunitomi M, Makino H, Kira S and Fujii M: Changes in serum leptin concentrations in overweight Japanese men after exercise. Diabetes Obes Metab (2004) 6: 332-337.
20. Miyatake N, Nonatak K and Fujii M: A new air displacement plethysmograph for the determination of Japanese body composition. Diabetes Obes Metab (1999) 1: 347-351.
21. McCrory MA, Gomez TD, Bernauer EM and Mole PA: Evaluation of a new air displacement plethysmograph for measuring human body composition. Med Sci Sports Exerc (1995) 27: 1686-1691.
22. Gordon T, Castelli WP, Hjortland MC, Kannel WB and Dawber TR: High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. Am J Med (1977) 62: 707-714.
23. 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: 982-92.
24. The Examination Committee of Criteria for 'Obesity Disease' in Japan; Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. Circ J (2002) 66: 987-992.
25. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA and Stern MP: Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes (1992) 41: 715-722.
26. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR and Groop L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care (2001) 24: 683-689.
27. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J and Salonen JT: The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA (2002) 288: 2709-2716.
28. 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.
29. Jin Y, Moriya T, Tanaka K, Matsubara M and Fujita Y: Glomerular hyperfiltration in non-proteinuric and non-hypertensive Japanese type 2 diabetic patients. Diabetes Res Clin Prac (2006) 71: 264-271.

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Short Communication

Decreasing Systolic Blood Pressure Is Associated with Improving Estimated Glomerular Filtration Rate (eGFR) with Lifestyle Modification in Japanese Healthy Women

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The link between changes in a subject's metabolic syndrome components and her estimated glomerular filtration rate (eGFR) was evaluated in healthy Japanese women. We used data for 53 Japanese women (46.0 ± 10.9 years) with a 1-year follow up. eGFR was defined by a new equation developed for Japan. There were no significant relationships between eGFR and clinical parameters at baseline. Subjects were given advice for dietary and lifestyle improvement. At the 1-year follow up, eGFR was significantly increased. In addition, changes in eGFR were weakly correlated with systolic blood pressure ($r = -0.306$, $p = 0.0260$). A decrease in systolic blood pressure may be associated with improving eGFR in Japanese women.

Key words: systolic blood pressure, estimated glomerular filtration rate (eGFR), metabolic syndrome, lifestyle modification

Chronic kidney disease (CKD) is a common disorder and has become a public health challenge [1]. For example, about 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, and 4.1% have moderate or severe CKD [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]. However, whether decreases in metabolic syndrome components are beneficial for improving eGFR, and what effects this has on eGFR remain to be investigated in a longitudinal study.

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

Subjects and Methods

Subjects. We used data for 53 Japanese women, aged 46.0 ± 10.9 years, who met the following criteria: (1) received a health check-up including 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).

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

	Baseline	Follow up	p
Number of Subjects	53		
Age	46.0 ± 10.9		
Height (cm)	156.1 ± 4.3		
Body weight (kg)	62.4 ± 8.8	60.9 ± 8.5	0.0002
Body mass index (kg/m^2)	25.6 ± 3.3	25.0 ± 3.3	0.0002
Abdominal circumference (cm)	78.7 ± 8.1	76.6 ± 8.3	0.0005
Systolic blood pressre (mmHg)	121.5 ± 14.1	119.8 ± 15.4	0.2772
Diastolic blood pressure (mmHg)	76.2 ± 9.4	74.6 ± 10.8	0.2245
Triglyceride (mg/dl)	98.2 ± 65.8	95.8 ± 62.1	0.7065
HDL cholesterol (mg/dl)	64.5 ± 14.5	64.6 ± 14.9	0.9362
Blood sugar (mg/dl)	95.1 ± 9.4	93.8 ± 9.3	0.2018
Cr (mg/dl)	0.58 ± 0.09	0.56 ± 0.10	0.0148
eGFR (ml/min/1.73m^2)	90.0 ± 17.9	94.2 ± 19.9	0.0215

Mean ± SD

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 amount of steps walked.

Ethical approval for the study was obtained from the Ethical Committee of 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 [5].

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 plasma glucose. eGFR was calculated using the following equation: eGFR (ml/min/1.73m^2) = $194 \times Cr^{-1.094} \times Age^{-0.287} \times 0.739$ (a constant derived specifically for women) [3]. Reduced eGFR was defined as an eGFR < 60 ml/min/1.73 m^2.

Definition of metabolic syndrome. Women with an abdominal circumference in excess of 90 cm were defined as having metabolic syndrome if they also had 2 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 ≥ 130/85 mmHg, 3) Impaired glucose tolerance: fasting plasma glucose ≥ 110 mg/dl [5].

Statistical analysis. Data are expressed as means ± standard deviation (SD). A statistical analysis was performed using a paired *t* 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 con-

tinuous variables; stepwise multiple regression analysis was also used.

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 decreased and eGFR was significantly increased. No subject was diagnosed as having metabolic syndrome and only one subject was diagnosed with reduced eGFR from baseline to the 1-year follow up. In addition, 2 subjects were identified as positive (+) for proteinuria at baseline and 4 subjects were identified as trace positive at the 1-year follow up.

The relationship between eGFR and clinical parameters at baseline was evaluated. There were no significant relationships between eGFR and other clinical parameters at baseline (Table 2).

Table 2 Simple correlation analysis between eGFR and clinical parameters at baseline

	r	p
Body weight (kg)	0.082	0.5594
Body mass index (kg/m^2)	0.033	0.8165
Abdominal circumference (cm)	−0.154	0.2708
Systolic blood pressre (mmHg)	−0.167	0.2333
Diastolic blood pressure (mmHg)	−0.119	0.3958
Triglyceride (mg/dl)	0.123	0.3785
HDL cholesterol (mg/dl)	−0.063	0.6566
Blood sugar (mg/dl)	−0.193	0.1662

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

	r	p
Body weight (kg)	0.188	0.1775
Body mass index (kg/m^2)	0.181	0.1945
Abdominal circumference (cm)	0.253	0.0672
Systolic blood pressre (mmHg)	−0.306	0.0260
Diastolic blood pressure (mmHg)	−0.112	0.4325
Triglyceride (mg/dl)	0.095	0.5006
HDL cholesterol (mg/dl)	0.227	0.1015
Blood sugar (mg/dl)	−0.214	0.1232

We further evaluated the relationship between changes in eGFR and changes in clinical parameters. Changes in eGFR were weakly correlated with changes in systolic blood pressure ($r = -0.306$, $p = 0.0260$) (Table 3, Fig. 1). However, changes in eGFR were not significantly correlated with changes in other metabolic components. We also used stepwise multiple regression analysis to evaluate the effect of changes in clinical parameters, *i. e.* body weight, BMI, abdominal circumference, systolic blood pressure, diastolic blood pressure, triglyceride, HDL cholesterol and blood sugar, on the change in eGFR, and found that only change in systolic blood pressure was significant [Change in eGFR = 3.632 − 0.349 (change in systolic blood pressure), $r^2 = 0.093$, $p = 0.0260$].

Finally, we further investigated the difference of change in eGFR between subjects who had different levels of systolic blood pressure at baseline [Group L, systolic blood pressure < 140 mmHg; Group H, systolic blood pressure ≥ 140 mmHg]. The changes in systolic blood pressure in Group H subjects (−1.20 ml/min/1.73 m^2) was lower than that in Group L subjects (4.9 ml/min/1.73 m^2) after 1 year, but not at a significant level ($p = 0.2822$).

Discussion

The main objective of this study was to explore the link between changes in eGFR and changes in metabolic syndrome components in Japanese women with a

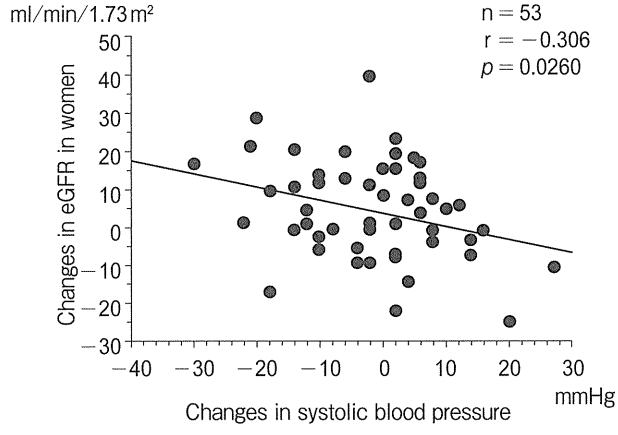

Fig. 1 Simple correlation analysis between changes in eGFR and changes in systolic blood pressure at 1-year follow up.

1-year follow up.

Tanaka *et al.* [6], Ninomiya T *et al.* [7] and Iseki *et al.* [8] reported that metabolic syndrome, using the modified ATP III definition [9], was associated with CKD in the Japanese population. Compared with subjects with 0 or 1 component 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, no subject was diagnosed as having metabolic syndrome, using the Japanese criteria, either at baseline or at the 1-year follow up. We have previously reported that the prevalence of metabolic syndrome was 3.6% in Japanese women [10]. However, with lifestyle modification after the initial health check-up, eGFR was significantly increased even in women without metabolic syndrome at the 1-year follow-up.

Hypertension contributes to the development of renal injury and end-stage renal disease [11-15]. Even high-normal blood pressure has been shown to be significantly associated with development of CKD in both sexes. Yamagata *et al.* 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 10-year follow up [11]. Tozawa *et al.* also reported a relative risk of 1.34 for end-stage renal failure for every increase of 10 mmHg in systolic blood pressure in 51,878 women investigated [12]. In the present study, there was no significant relationship between eGFR and systolic blood pressure at baseline. However, we revealed that, with lifestyle modification, changes in systolic blood pressure were correlated with changes in eGFR in women without metabolic syndrome. Therefore, the clinical impact of hypertension was noted.

Potential limitations remain in our study. First, the small sample size in our study makes it difficult to infer causality between eGFR and hypertension. Second, we also could not reveal the mechanism of the linkage between eGFR and hypertension. Further prospective studies are needed in Japanese subjects. Third, most of the enrolled subjects were not diagnosed as CKD at baseline. Therefore, the results in this study may not apply for patients with CKD.

In conclusion, a decrease in systolic blood pressure with lifestyle modification was associated with an increase in eGFR. Therefore, lifestyle modification may be a necessary and useful measure for the prevention of CKD.

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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. Miyatake 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. 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).
6. 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.
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. 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.
9. 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.
10. 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.
11. 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.
12. Tozawa M, Iseki K, Iseki C, Kinjo K, Ikemiya Y and Takishita S: Blood pressure predicts risk of developing end-stage renal disease in men and women. Hypertension (2003) 41: 1341-1345.

13. Klag MJ, Whelton PK, Randall BL, Neaton JD, Brancati FL, Ford CE, Shulman NB and Stamler J: Blood pressure and end-stage renal disease in men. N Engl J Med (1996) 334: 13-18.
14. Klag MJ, Whelton PK, Randall BL, Neaton JD, Brancati FL and Stamler J: End-stage renal disease in African-American and white men. 16-year MRFIT findings. JAMA (1997) 277: 1293-1298.
15. Reynolds K, Gu D, Muntner P, Kusek JW, Chen J, Wu X, Duan X, Chen CS, Klag MJ, Whelton PK and He J: A population-based, prospective study of blood pressure and risk for end-stage renal disease in China. J Am Soc Nephrol (2007) 18: 1928-1935.

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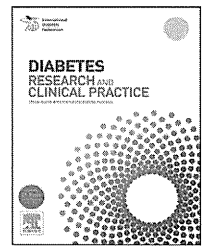

Microinflammation is a common risk factor for progression of nephropathy and atherosclerosis in Japanese patients with type 2 diabetes

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ABSTRACT

Aim: This study aimed to evaluate the change of serum levels of proinflammatory molecules in patients with type 2 diabetes and clarify the involvement of these molecules in diabetic nephropathy and atherosclerosis.

Methods: Sixty-six Japanese type 2 diabetic patients (T2DM) and 39 healthy control subjects were enrolled. We assessed clinical parameters, urinary albumin excretion rate (AER), brachial-ankle pulse wave velocity (baPWV), intima media thickness (IMT) and serum levels of proinflammatory molecules.

Results: Serum levels of IL-6, IP-10 and MCP-1 were significantly higher in T2DM than in control subjects. In T2DM, serum levels of high-sensitivity (hs) CRP, IP-10, hsTNF-α, VCAM-1 and E-selectin were positively correlated with AER. Serum levels of IP-10, hsTNF-α and VCAM-1 were positively correlated with baPWV. Serum levels of hsCRP, IL-6, IP-10 and hsTNF-α were positively correlated with IMT. Multiple linear regression analysis revealed that serum levels of hsTNF-α were independently associated with AER ($\beta = 0.235$, $P = 0.038$) and serum levels of IP-10 were independently associated with baPWV ($\beta = 0.209$, $P = 0.047$) and IMT ($\beta = 0.303$, $P = 0.032$).

Conclusion: Our results suggest that low-grade inflammation, microinflammation, may be a common risk factor for diabetic nephropathy and atherosclerosis in Japanese type 2 diabetic patients.

1. Introduction

Microinflammation, low-grade inflammation occurred on the vascular wall, is involved in the mechanism of atherosclerosis [1]. The elevation of serum C-reactive protein (CRP) level is known to be a risk factor for ischemic heart disease [2,3]. Proinflammatory molecules, such as interferon-γ inducible protein (IP)-10 are reported to be involved in the formation of atherosclerotic lesion [4]. Microinflammation is also occurred in patients with diabetes. Several reports indicated that CRP [5] and proinflammatory cytokines including interleukin (IL)-6 and tumor necrosis factor (TNF)-α [6], are elevated in patients with type 2 diabetes. Elevated levels of CRP and IL-6 predict insulin resistance and development of type 2 diabetes [7,8].

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The mechanisms for elevation of serum levels of proinflammatory molecules in type 2 diabetes remain unclear, although it might be at least partially caused by oxidative stress [9]. Activation of nuclear factor-κB through oxidative stress-induced by hyperglycemia increases concentrations of circulating proinflammatory cytokines [10].

We previously demonstrated that leukocyte adhesion molecules including E-selectin and P-selectin are up-regulated in the kidney of diabetic patients [11]. We also reported that intercellular adhesion molecules (ICAM)-1 is up-regulated in the kidney of experimental diabetic rats and mediates infiltration of macrophages into diabetic kidney [12]. In another report, serum level of vascular cell adhesion molecules (VCAM)-1 is elevated in diabetic patients [13]. Moreover, macrophage infiltration and renal injuries were prevented in ICAM-1 deficient mice after induction of diabetes [14]. These findings suggest that inflammatory process is also involved in the pathogenesis of diabetic nephropathy.

We have recently reported that serum level of IL-18 is positively correlated with urinary albumin excretion, brachial-ankle pulse wave velocity (baPWV) and intima media thickness of carotid artery (IMT) and both serum and urinary levels of IL-18 are independently correlated with urinary albumin excretion rate (AER) [15]. These findings suggest that IL-18 might be a predictor of progression of diabetic nephropathy as well as atherosclerosis. Elevated level of fibrinogen, an independent risk factor for cardiovascular disease, is also associated with AER [16]. These findings suggest that inflammatory process is involved in both atherosclerosis and diabetic nephropathy. Proinflammatory cytokines, chemokines and adhesion molecules may compose a complex network and contribute to the progression of vascular complications in diabetic patients.

The aim of this study is to evaluate the change of serum profile of proinflammatory molecules including cytokines, chemokines and adhesion molecules in patients with type 2 diabetes and clarify the involvement of these molecules in diabetic nephropathy and atherosclerosis.

2. Subjects, materials and methods

2.1. Study population

A total of 66 Japanese patients (32 females and 34 males) with type 2 diabetes were enrolled in this study. All patients were fulfilled the following inclusion criteria; initially diagnosed diabetes at over 40 of their age, negative about antibody to GAD, no history of ketoacidosis, no renal insufficiency (creatinine clearance >1.00 ml/s), never received any hormone replacement therapy. Cardiovascular disease was defined as an attack of stroke, ischemic heart disease and arteriosclerosis obliterans. Their mean age was 61.0 ± 7.8 (mean ± SD), diabetic duration 10.4 ± 6.4 years, BMI 23.9 ± 3.2 kg/m^2, HbA_{1c} 7.3 ± 1.2%.

A total of 39 age and sex matched healthy adults (24 females and 15 males) were enrolled as control subjects. Inclusion criteria for control subjects was as follows; never diagnosed diabetes, fasting plasma glucose <6.1 mmol/l, HbA_{1c} <5.8%, blood pressure <140/90 mmHg, AER <30 mg/gCr, creatinine clearance >1.00 ml/s, no medication or treatment. Their mean age was 58.1 ± 7.6, BMI 22.1 ± 2.3 kg/m^2, HbA_{1c} 5.1 ± 0.5%.

Informed consent was obtained from all participants and control subjects, and this study was approved by the Ethical Committee of Okayama Saiseikai General Hospital.

2.2. Collection of blood and urine samples

Collection of blood and urine samples was performed in the early morning after overnight fast. Creatinine clearance was determined using Cockcroft-Gault formula. AER was determined with immunoturbidimetric assay Micro Alb (Nitto Boseki Co., Ltd, Tokyo, Japan). Normoalbuminuria was defined as AER 30 mg/gCr, microalbminuria was defined as AER 30–299 mg/gCr and macroalbuminuria was defined as AER >300 mg/gCr. Blood and urine samples were centrifuged immediately after collection and the supernatants were stored at −80 °C and −30 °C until analysis, respectively. All samples were measured at one time after sample collection.

2.3. Measurements of serum proinflammatory molecules

Serum levels of IL-6 were measured using a chemiluminescent enzyme assay (CLEIA kit; Fujirebio, Tokyo, Japan). Serum levels of high-sensitivity (hs) TNF-α, IP-10, monocyte chemotactic protein (MCP)-1, ICAM-1, VCAM-1, E-selectin and L-selectin were measured using a quantitative sandwich enzyme immunoassay (R&D Systems, Minneapolis, USA). Serum P-selectin was measured using an enzyme-linked immunosorbent assay (TaKaRa, Kyoto, Japan).

2.4. Measurements of carotid IMT and baPWV

IMT of the common carotid artery was determined using duplex ultrasonography with a 7.5 MHz linear transducer (SSD-5500; Aloka, Tokyo, Japan). As we previously referred [15], carotid IMT was defined as the distance from the leading edge of the first echogenic line to the second echogenic line on a sonographic image. Measurements of IMT were made at each of the three sites of the greatest thickness on both sides. The mean of these maximal IMT measurements was used for analysis.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice with the patient in a sitting position after 5 min rest. Left and right baPWV was measured automatically using an ABI-form (BP-203RPE II; Nippon Colin, Komaki, Japan). A trained physician at Okayama Saiseikai General Hospital performed all scans. In this study, the highest values of SBP, DBP, IMT and baPWV from the left and the right sides were used for the evaluation of each patient.

2.5. Statistics

All statistical analysis was performed by SPSS for Windows statistical software system. All data are represented as mean ± SD or actual numbers. Comparison of data between type 2 diabetic patients and control subjects was evaluated using unpaired Student's t-test and χ^2 for sex. AER, hsCRP, IL-6, hsTNF-α, IP-10, MCP-1, ICAM-1, VCAM-1, P-selectin, E-selectin and L-selectin were not normally distributed (Shapiro–Wilkes test) and natural logarithmic transformation was used to shift

the variables into normal distribution. Correlation was evaluated using univariate and multivariate linear regression analysis. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. *Clinical characteristics and serum levels of proinflammatory molecules in diabetic patients and control subjects*

Clinical characteristics and serum levels of proinflammatory molecules between type 2 diabetic patients and control subjects were shown in Table 1. BMI, HDL-cholesterol, fibrinogen, hsCRP in type 2 diabetic patients were significantly higher than in control subjects. As for blood pressure, no significant valiance was observed between type 2 diabetic patients and control subjects. In type 2 diabetic patients, the number of patients with normoalbuminuria, microalbuminuria and macroalbuminuria was 42, 18 and 6, respectively. AER, baPWV and IMT in type 2 diabetic patients were significantly higher than in control subjects (AER 120 ± 480 vs. 10.2 ± 10 mg/gCr, $P < 0.001$; baPWV 17.0 ± 3.5 vs. 14.7 ± 2.5 m/s, $P < 0.001$; IMT 0.86 ± 0.19 vs. 0.73 ± 0.15 mm, $P = 0.001$). There were 11 diabetic patients with episodes of cardiovascular disease. In type 2 diabetic patients, 8 patients received dietary therapy only, 31 patients received oral hypoglycemic agents and 27 patients received insulin replacement therapy. Twenty-four patients were administered angiotensin converting enzyme inhibitors or angiotensin receptor antagonists. Fourteen patients were administered statins.

Serum IL-6 levels were significantly higher in type 2 diabetic patients than in control subjects (1.92 ± 1.24 vs. 1.32 ± 0.78 pg/ml, $P = 0.005$). There was no significant difference in TNF-α levels between type 2 diabetic patients and control subjects. Serum IP-10 levels were significantly higher in type 2 diabetic patients than in control subjects (76.5 ± 37 vs. 55.7 ± 16 pg/ml, $P = 0.004$).

Table 1 – Characteristics of type 2 diabetic patients and control subjects.

	Control subjects ($n = 39$)	Diabetic patients ($n = 66$)	P-value
Sex (female) (n)	24	32	0.228
Age (years)	58.1 ± 7.6	61.0 ± 7.8	0.062
BMI (kg/m^2)	22.1 ± 2.3	23.9 ± 3.2	0.001
SBP (mmHg)	125 ± 12	130 ± 15	0.064
DBP (mmHg)	77 ± 10	77 ± 9	0.954
Fasting plasma glucose (mmol/l)	5.46 ± 0.5	8.44 ± 3.3	<0.001
HbA_{1c} (%)	5.1 ± 0.5	7.3 ± 1.2	<0.001
Creatinine clearance (ml/s)	1.53 ± 0.3	1.55 ± 0.4	0.811
Total cholesterol (mmol/l)	5.69 ± 1.0	5.37 ± 0.8	0.082
HDL-cholesterol (mmol/l)	1.57 ± 0.5	1.39 ± 0.4	0.044
LDL-cholesterol (mmol/l)	3.47 ± 0.9	3.25 ± 0.8	0.180
Triglyceride (mmol/l)	1.27 ± 0.6	1.59 ± 1.1	0.101
Fibrinogen (g/l)	2.76 ± 0.4	3.01 ± 0.5	0.013
hsCRP (mg/l)	0.054 ± 0.06	0.11 ± 0.1	–
(ln)hsCRP (ln[mg/l])	−3.28 ± 1.2	−2.69 ± 0.9	0.006
Duration of diabetes (years)	–	10.4 ± 6.4	–
History of cardiovascular events (yes) (n)	–	11	–
Albumin excretion rate (mg/gCr)	10.2 ± 10	120 ± 480	–
(ln)AER (ln[mg/gCr])	1.92 ± 0.9	2.92 ± 1.6	<0.001
baPWV (m/s)	14.7 ± 2.5	17.0 ± 3.5	<0.001
IMT (mm)	0.73 ± 0.15	0.86 ± 0.19	0.001
Serum IL-6 (pg/ml)	1.32 ± 0.78	1.92 ± 1.24	–
(ln) serum IL-6 (ln[pg/ml])	0.12 ± 0.58	0.47 ± 0.63	0.005
Serum hsTNF-α (pg/ml)	1.18 ± 0.46	1.23 ± 0.48	–
(ln) serum hsTNF-α (ln[pg/ml])	0.06 ± 0.54	0.15 ± 0.37	0.363
Serum IP-10 (pg/ml)	55.7 ± 16	76.5 ± 37	–
(ln) serum IP-10 (ln[pg/ml])	3.98 ± 0.27	4.25 ± 0.40	0.004
Serum MCP-1 (pg/ml)	213 ± 50	249 ± 57	–
(ln) serum MCP-1 (ln[pg/ml])	5.33 ± 0.26	5.49 ± 0.23	0.001
Serum ICAM-1 (ng/ml)	225 ± 66	236 ± 62.1	–
(ln) serum ICAM-1 (ln[ng/ml])	4.39 ± 0.62	5.42 ± 0.28	<0.001
Serum VCAM-1 (ng/ml)	651 ± 181	701 ± 181	–
(ln) serum VCAM-1 (ln[ng/ml])	6.44 ± 0.27	6.52 ± 0.25	0.151
Serum P-selectin (ng/ml)	230 ± 96	243 ± 119	–
(ln) serum P-selectin (ln[ng/ml])	5.33 ± 0.26	5.37 ± 0.51	0.583
Serum E-selectin (ng/ml)	45.2 ± 21	47.2 ± 18	–
(ln) serum E-selectin (ln[ng/ml])	3.72 ± 0.42	3.78 ± 0.38	0.434
Serum L-selectin (ng/ml)	863 ± 222	799 ± 208	–
(ln) serum L-selectin (ln[ng/ml])	6.73 ± 0.26	6.65 ± 0.26	0.140

Data are mean ± SD. P for type 2 diabetic subjects versus control subjects.

Table 2 – Simple correlation between logarithmic AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules in Japanese type 2 diabetic patients.

Variables	(ln)AER		baPWV		IMT	
	r	P	r	P	r	P
Sex (female) (n)	0.023	0.857	0.079	0.533	0.235	0.062
Age (years)	0.222	0.073	0.627	<0.001	0.242	0.054
BMI (kg/m^2)	0.161	0.198	0.191	0.130	0.231	0.066
SBP (mmHg)	0.229	0.064	0.323	0.009	−0.006	0.960
DBP (mmHg)	0.096	0.442	0.135	0.286	0.060	0.637
Fasting plasma glucose (mmol/l)	0.187	0.133	0.032	0.803	0.045	0.721
HbA_{1c} (%)	0.282	0.022	0.199	0.115	0.184	0.145
Creatinine clearance (ml/s)	−0.081	0.519	−0.095	0.458	0.058	0.653
Total cholesterol (mmol/l)	0.046	0.715	−0.028	0.828	−0.053	0.676
HDL-cholesterol (mmol/l)	−0.120	0.343	−0.024	0.855	−0.374	0.003
LDL-cholesterol (mmol/l)	−0.012	0.923	−0.088	0.492	0.163	0.198
Triglyceride (mmol/l)	0.203	0.103	0.057	0.656	0.103	0.420
(ln) hsCRP (ln[mg/l])	0.263	0.037	0.100	0.442	0.296	0.021
(ln) serum IL-6 (ln[pg/ml])	0.020	0.874	0.013	0.920	0.298	0.017
(ln) serum hsTNF-α (ln[pg/ml])	0.329	0.010	0.307	0.018	0.332	0.011
(ln) serum IP-10 (ln[pg/ml])	0.371	0.002	0.509	<0.001	0.265	0.036
(ln) serum MCP-1 (ln[pg/ml])	0.090	0.471	0.152	0.229	0.107	0.401
(ln) serum ICAM-1 (ln[ng/ml])	0.203	0.101	0.040	0.757	−0.016	0.900
(ln) serum VCAM-1 (ln[ng/ml])	0.341	0.005	0.331	0.008	−0.028	0.826
(ln) serum P-selectin (ln[ng/ml])	0.101	0.418	0.020	0.876	0.227	0.071
(ln) serum E-selectin (ln[ng/ml])	0.444	<0.001	0.018	0.889	0.156	0.218
(ln) serum L-selectin (ln[ng/ml])	−0.004	0.973	−0.006	0.960	−0.183	0.148

Data are mean ± SD. r, simple correlation coefficient.

Serum MCP-1 levels were significantly higher in type 2 diabetic patients than in control subjects (249 ± 57 vs. 213 ± 50 pg/ml, $P = 0.001$). Among adhesion molecules, significant difference was observed only in ICAM-1 levels between the two groups.

3.2. *Simple correlation between AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules in patients with type 2 diabetes*

We investigated simple correlation between clinical characteristics or proinflammatory molecules and AER, baPWV or IMT in type 2 diabetic patients. The correlation was shown in Table 2. A significant positive correlation between AER and HbA_{1c} (correlation coefficient [r] = 0.282, $P = 0.022$), serum levels of hsCRP ($r = 0.263$, $P = 0.037$), hsTNF-α ($r = 0.329$, $P = 0.010$), IP-10 ($r = 0.371$, $P = 0.002$), VCAM-1 ($r = 0.341$, $P = 0.005$) and E-selectin ($r = 0.444$, $P < 0.001$) was observed. BaPWV correlated positively with Age ($r = 0.627$, $P < 0.001$), SBP ($r = 0.323$, $P = 0.009$), serum levels of hsTNF-α ($r = 0.307$, $P = 0.018$), IP-10 ($r = 0.509$, $P < 0.001$) and VCAM-1 ($r = 0.331$, $P = 0.008$). IMT correlated positively with HDL-cholesterol ($r = -0.374$, $P = 0.003$), serum levels of hsCRP ($r = 0.296$, $P = 0.021$), IL-6 ($r = 0.298$, $P = 0.017$), hsTNF-α ($r = 0.332$, $P = 0.011$) and IP-10 ($r = 0.265$, $P = 0.036$).

As previously reported [17,18], significant simple correlation between AER, baPWV and IMT was observed both in all participants and in type 2 diabetic patients (data not shown).

3.3. *Multiple linear regression analysis of relationships between AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules*

Serum levels of hsTNF-α and IP-10 were simply correlated with AER, baPWV and IMT. We next performed multiple linear regression analysis for relationships between AER, baPWV or IMT and hsTNF-α, IP-10 and clinical parameters that were significantly correlated with AER, baPWV or IMT. The correlation was shown in Table 3. Previous history of

Table 3 – Multiple linear regression analysis of relationships between AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules.

Variables	β	P
Dependent variable: (ln)AER, $R^2 = 0.498$, $P < 0.001$		
Independent variable		
Previous history of cardiovascular events	0.549	<0.001
ln serum hsTNF-α	0.235	0.038
ln hsCRP	0.137	0.265
ln serum IP-10	0.127	0.230
HbA_{1c}	0.062	0.575
Dependent variable: baPWV, $R^2 = 0.538$, $P < 0.001$		
Independent variable		
Age	0.398	<0.001
SBP	0.227	0.035
Duration of diabetes	0.210	0.041
ln serum IP-10	0.209	0.047
ln serum hsTNF-α	0.118	0.260
Dependent variable: IMT, $R^2 = 0.249$, $P = 0.016$		
Independent variable		
ln serum IP-10	0.303	0.032
ln serum hsTNF-α	0.144	0.316
HDL-cholesterol	−0.155	0.328
ln hsCRP	0.052	0.734
ln serum IL-6	0.049	0.774

β, standard correlation coefficients; R^2, multiple coefficients of determination.