diabetes, and this hypothesis should be tested in a future clinical study.

Study limitation

There was a notable limitation in the present study. Although the limited sensitivity in the detection of insulin resistance, we tried to look at the maximum response and used the highest concentration of MGO as possible. In the present study, 4-week treatment with 1% MGO in drinking water increased plasma MGO level by about 7.5-fold compared with the control rats in Sprague–Dawley rats (data not shown). The increased magnification of plasma MGO level in the present study is as high as that in patients with chronic kidney disease (CKD) stages 5 compared with the healthy controls (about 5–10 fold) [36]. With the technical limitation of measurements and the control of plasma and/or tissue concentration of MGO, we were not able to determine whether MGO on insulin resistance or salt sensitivity were dose dependent. Further investigation is required in the future study by amelioration of these techniques.

In summary, we have demonstrated that MGO induces insulin resistance as well as salt sensitivity of blood pressure in normotensive Sprague–Dawley rats. Our results suggest that these effects of MGO may be mediated at least in part by increased oxidative stress or AGEs formation, or both. Our present study provides further evidence that MGO is one of the causative factors in the pathogenesis of insulin resistance and salt-sensitive hypertension. Antioxidants and ACE inhibitors may be useful for the treatment of chronic kidney disease individuals with insulin resistance and salt sensitivity.

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Letter

Carbonated soft drinks and carbonyl stress burden

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ABSTRACT — Carbonated soft drinks reportedly contain methylglyoxal (MG), which is strongly associated with human carbonyl stress. We sought to evaluate the effects of carbonated drink intake on human carbonyl stress. We measured MG levels in 4 commercial beverage brands, and evaluated the changes in plasma MG in healthy subjects following the intake of carbonated drinks. By 30 min after intake of samples containing high glucose and high MG, the levels of plasma MG, glucose, insulin and uric acid had increased significantly, and then returned to basal levels by 120 min. After intake of the low-calorie carbonated samples containing little MG, there were no increases in plasma MG. Our results suggest that glucose-containing carbonated soft drinks are associated with increases in not only glucose but also carbonyl burden.

Key words: Carbonated soft drink, MG, Carbonyl stress

INTRODUCTION

Carbonated soft drink consumption reportedly leads to cardiometabolic risk factors, such as hypertension, impaired glucose tolerance and chronic kidney disease (Dhingra *et al.*, 2007; Winkelmayer *et al.*, 2005; Saldana *et al.*, 2007). Moreover, previous reports have shown that carbonated drinks contain methylglyoxal (MG), which is a highly reactive carbonyl compound and major precursor of advanced glycation end products (AGEs), and displays toxicity in cells and tissues (Tan *et al.*, 2008; Fukunaga *et al.*, 2004; Okado *et al.*, 1996; Ramasamy *et al.*, 2006). Food and beverages represent exogenous sources of MG (Nemet *et al.*, 2006); however, few reports have evaluated the actual effects of drinking and eating such products on plasma MG levels.

Moreover, carbonyl stress caused by the accumulation of reactive carbonyl compounds is also associated with hypertension, diabetic complications and uremic states, and carbonyl stress plays a pathological role in these diseases (Wang *et al.*, 2008; Beisswenger *et al.*, 2003; Miyata *et al.*, 2001; Nakayama *et al.*, 2008). Therefore, whether the intake of carbonated soft drinks affects the carbonyl stress burden is of clinical importance.

In this study, we measured MG levels in 4 commercial beverage brands, and evaluated the changes in plasma MG levels and metabolic factors, such as glucose and uric acid (UA), after intake of 2 types of carbonated soft drink (regular and low-calorie).

MATERIALS AND METHODS

Beverage samples

We purchased 4 commercially available types of carbonated soft drink, including cola, lemon-lime soft drink, and 2 brands of diet cola (Table 1). Samples A (a cola) and C (a diet cola) were used for the loading tests.

Subjects

Subjects comprised 6 healthy volunteers (age range, 20 to 48 years) for loading tests with sample A, and 5 volunteers (age range, 22 to 48 years) for sample C. All subjects had normal renal function and no metabolic risk factors. After an 8-hr overnight fast, blood samples were obtained (pre) and subjects then consumed 300 ml of sample A or 500 ml of sample C. Blood samples were drawn after 30,

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Table 1. Concentrations of methylglyoxal in 4 types of carbonated drink

Sample	Category	Carbohydrate (g/100 ml)	MG[a] (μM)
A	Cola	11.3	7.2
B	Lemon-lime soft drink	10.0	5.8
C	Diet-type cola	0.0	0.2
D	Diet-type cola	0.0	0.7

[a] MG, methylglyoxal

60 and 120 min. The Ethics Committee of Tohoku University approved this study protocol, and informed consent was obtained from all subjects.

Laboratory analyses

After centrifugation of blood, plasma was aspirated and stored at −80°C until assayed. MG levels were assayed by derivatization with *o*-phenylenediamine (*o*-PD) and electrospray ionization liquid chromatography mass spectrometry (ESI/LC/MS) of the resulting quinoxalines, as reported previously (Nakayama *et al.*, 2008). To obtain more precise data, we modified the analytical conditions of LC/MS. The gradient speed of the mobile phase was slowed (from 6 to 10 min) and the mass/charge ratio (*m/z*) was detected more precisely (from *m/z* 145 to *m/z* 145.07). The resulting plasma MG levels from this new method were lower than our previous data derived from the previous method, but high relativity between the new and old methods was observed for 30 plasma samples: 10 from healthy controls and 20 from patients with renal failure (regression equation: $y = 0.89x - 92$, $R^2 = 0.97$). Plasma insulin was measured by the chemiluminescent enzyme immunoassay method, and glucose and other laboratory data were measured using an automatic analyzer at our clinical laboratory. Chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Statistical analyses

SPSS version 11.0 software (SPSS Japan, Tokyo, Japan) was used to evaluate the changes in plasma before and after soft drink intake by analysis of variance with repeated measures and Dunnett's test. Values of $P < 0.05$ were considered to indicate statistical significance.

RESULTS AND DISCUSSION

The MG concentrations in 4 types of carbonated soft drink are listed in Table 1. Samples A and B contained high concentrations of carbohydrates and significant levels of MG. Samples C and D were low-calorie drinks and contained very low MG levels.

We examined the changes in plasma MG levels after intake of sample A, which contained high glucose levels and the highest level of MG among the 4 drinks, and after intake of sample C, a diet-type drink. As shown in Fig. 1, plasma MG, glucose and insulin were increased in most subjects at 30 min after intake of sample A, while no remarkable change was observed in the test for sample C, except that the glucose level gradually increased. The increases in plasma MG at 30 min after intake of sample A were statistically significant, indicating that glucose-containing carbonated soft drinks may, at least partly, increase the carbonyl burden. In some subjects (4 and 5), the increases in both plasma MG and glucose were observed at 30 min (Figs. 1a and c), and these results may suggest that the increases in MG was due to secondary production from absorbed glucose. However, in other subjects (2 and 3), plasma MG was higher at 30 min without a concomitant increase in plasma glucose. Moreover, the amount of MG contained in sample A was 2.2 μmol (7.2 μmol/l, 300 ml), which was sufficient to raise the concentration from 110 to 170 nmol/l in 36 l water, which is similar to the body fluid volume in a person weighing 60 kg. Therefore, the increase in plasma MG was most likely due to direct absorption from the drinks. A previous report showed that significantly high levels of MG (from 3.3 to 19.3 μM) were present in 11 brands of carbonated soft drinks (Tan *et al.*, 2008), and their findings are coincident with the results of the present study.

In addition, UA levels were slightly but significantly, higher at 30 and 60 min. The extent of these changes was very small, and thus, its effect may be of little clinical significance. However, a high level of UA is reportedly one of the independent risk factors for cardiovascular disease (Choi and Curhan, 2007), therefore it is of interest whether plasma UA is associated with habitual intake of carbonated soft drinks.

Curiously, in all subjects, although the changes were small, plasma glucose levels gradually increased after intake of sample C, which contained no carbohydrate, while insulin levels did not increase. The threshold level of insulin secretion is thought to be above 100 mg/dl, and thus these increases in glucose were too small to stimulate beta cells to release insulin. These slow changes in glucose may be physiologic phenomena caused by circadian changes in hormones (e.g., insulin, glucagon and cortisol), but the exact mechanisms at work remains unclear.

In conclusion, glucose-containing carbonated soft

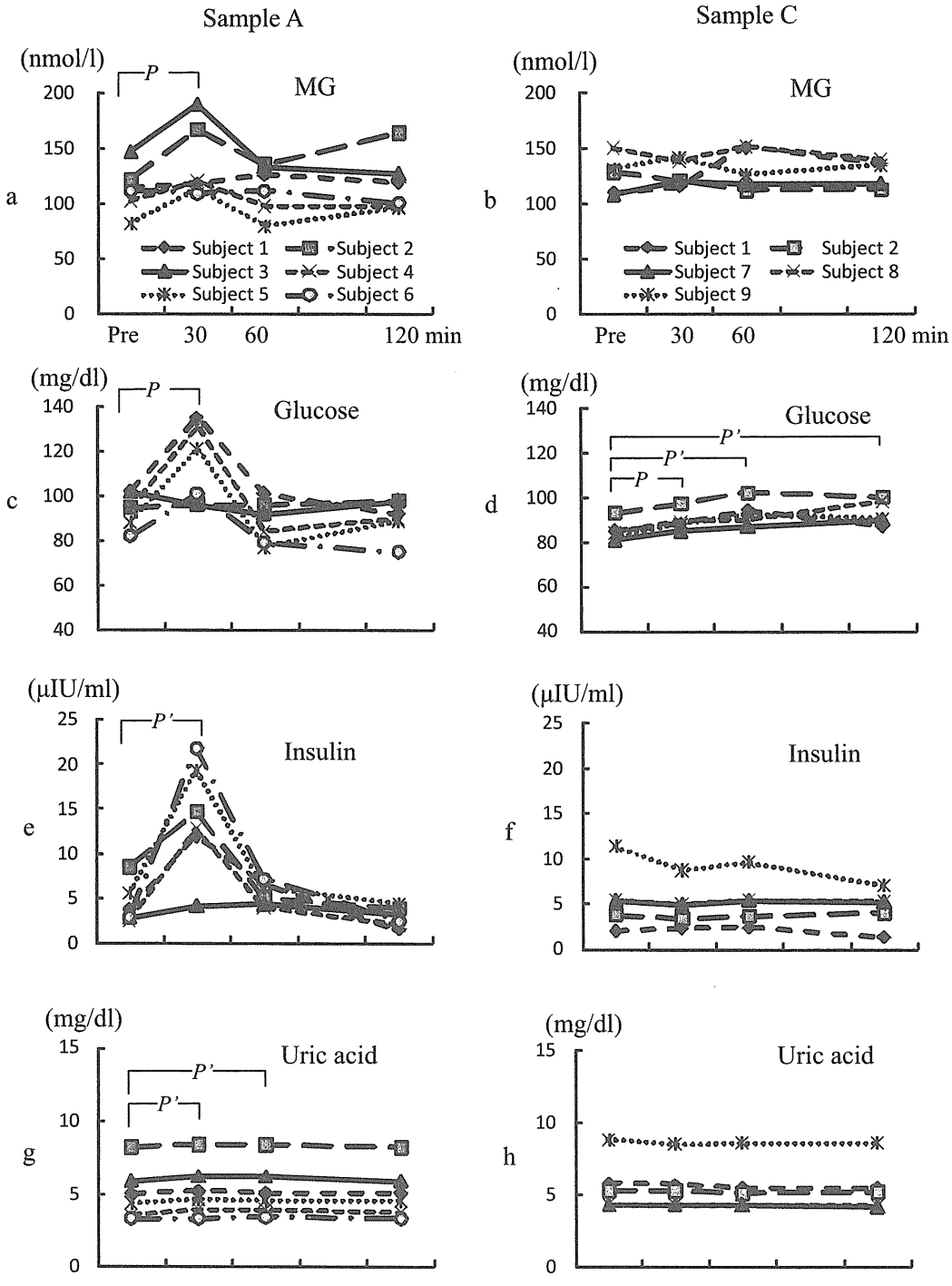

Fig. 1. Changes in plasma MG, glucose, insulin and UA levels before and after intake of sample A (glucose containing carbonated drink) (a, c, e, g) or sample C (diet-type carbonated drink) (b, d, f, h). Subjects 1 and 2 participated in both tests. For sample A, the MG level (mean ± S.D.) at pre and 30 min was 113 ± 22 and 136 ± 34 nM, respectively (a). The glucose level at pre and 30 min was 94 ± 8 and 113 ± 18 mg/dl, respectively (c). The insulin level at pre and 30 min was 4.4 ± 2.3 and 14.1 ± 6.2 μIU/ml, respectively (e). The UA level at pre, 30 and 60 min was 5.0 ± 1.8, 5.3 ± 1.8 and 5.3 ± 1.8 mg/dl, respectively (g). For sample C, the glucose level at pre, 30, 60 and 120 min was 85 ± 5, 89 ± 5, 93 ± 6 and 93 ± 6 mg/dl, respectively (d). $P < 0.05$ vs pre, $P' < 0.01$ vs pre.

drinks appear to lead to a transient increase in plasma MG levels. It is of great interest whether habitual intake of carbonated drinks enhances human carbonyl stress and UA levels, or is involved with enhanced cardiovascular events among these subjects. Further studies are required to address these issues.

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Biological Effects of Electrolyzed Water in Hemodialysis

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Key Words
Hemodialysis · Electrolyzed water · Oxidative stress · Methylglyoxal · Neutrophils

Abstract
Background/Aims: The application of electrolyzed water (EW) at the cathode side to manufacture reverse osmosis (RO) water and hemodialysis (HD) solution can actually lead to less oxidative capacity in chemical terms. The present study examined the biological actions of this water on human polymorphonuclear leukocytes (PMNs), and the clinical feasibility of applying this technology to HD treatment. ***Methods:*** RO water using EW (e-RO) exhibited less chemiluminescence in luminol-hydrogen peroxide and higher dissolved hydrogen levels (–99.0 ppb) compared with control RO water. The effects of e-RO on PMN viability were tested. HD using e-RO was performed for 12 consecutive sessions in 8 patients for the feasibility test. ***Results:*** Basal cellular viability and function to generate superoxide radicals of PMNs were better preserved by e-RO application. In the clinical trial, reductions of blood pressure were noted, but no adverse events were observed. There were no changes in the blood dialysis parameters, although methylguanidine levels were significantly decreased at the end of study. ***Conclusion:*** The present study demonstrated the capacity of e-RO to preserve the viability of PMNs, and the clinical feasibility of applying this water for HD treatment. The clinical application of this technology may improve the bio-compatibility of HD treatment.

Introduction

Evidence suggests that enhanced oxidative stress plays a crucial role in poor outcomes of patients on maintenance hemodialysis (HD) [1, 2]. In addition to a uremic milieu [1], several factors in the HD system have been found to be involved in the pathological mechanism of these poor outcomes, including bio-incompatibility of the dialysis membrane, contamination of the HD solution and loss of antioxidants during HD [3–7].

Part of this study was presented at the 2007 Annual Meeting of the American Society of Nephrology, San Francisco, Calif., USA.

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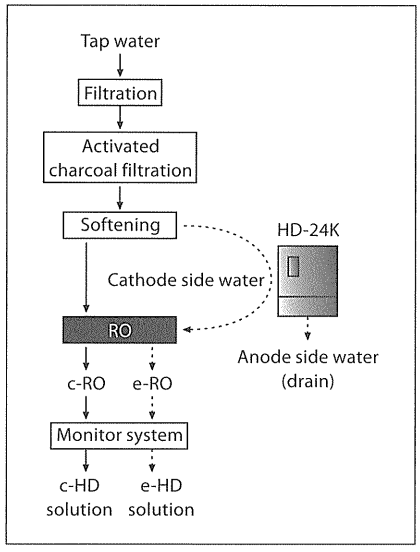

Fig. 1. Manufacturing process for HD solution using reverse osmosis water from the cathode-side of electrolyzed water. c-RO = control reverse osmosis water; e-RO = reverse osmosis water using electrolyzed water at the cathode side.

Water electrolysis renders 2 types of water: acidic water at the anode side and alkali water at the cathode side. It has been shown that alkali water (called electrolyzed water, EW) at the cathode side exhibits unique chemical properties, such as highly dissolved hydrogen with antioxidative capacity [8, 9]. An in vitro study showed that chemical reactions to generate superoxide anions and hydrogen peroxide (H_2O_2) were suppressed by EW treatment [8]. Based on these findings, therapeutic application of EW or water with highly dissolved hydrogen has been studied in animal models of diabetes [10, 11] and ischemic brain injury [12].

Furthermore, EW has been used to manufacture HD solution to reduce oxidative stress in patients [13–15]. To date, the limited clinical experience using this technology shows that EW suppresses oxidative/inflammatory markers in HD patients [13, 14]; however, most of the biological and clinical effects of EW remain unclear. The present study aimed to: (1) examine the biological action of EW in terms of whether it could ameliorate injury to human polymorphonuclear leukocytes, which may play a central role in excess inflammation or oxidative stress in HD patients [16], and (2) to test the clinical feasibility of applying this technology to HD treatment.

Materials and Methods

Manufacture of Test Solutions

Details of the manufacturing process were reported previously [15]. Briefly, test solutions were manufactured as follows (fig. 1): prefiltered water was processed by activated charcoal filtration and water softening to supply the water electrolysis system HD-24K (Nihon Trim, Osaka, Japan), where water was electrolyzed by direct current supply to the anode and cathode electrode plates. Water at the anode side was drained out, and EW was collected to supply the reverse osmosis equipment (MH500CX, Japan Water System Corp., Tokyo, Japan) at 500 ml/min. The intensity of the electrolysis was adjusted to maintain a pH $\leq$10.0. The reverse osmosis water made by EW (e-RO) was supplied to a personal HD monitoring system (DBB-22B, Nikkiso, Tokyo, Japan) to make the HD solution by mixing with a liquid dialysis solution concentrate.

The pH of the e-RO ranged from 9.0 to 10.0, with mean dissolved hydrogen levels of 99.0 ppb. The dissolved hydrogen level was detected by a gas analyzer (DH-35A hydrogen gas analyzer, Mitsuwa Rikagaku, Osaka, Japan). The hemodialysis solution made by e-RO (e-HD) did not differ from the control HD (c-HD) solution in respect of the electrolyte composition or pH; however, the former solution had a higher level of dissolved hydrogen (80 vs. 0 ppb).

Biological Effects of EW on Polymorphonuclear Leukocytes

Measurement of Cellular Viability

Human polymorphonuclear leukocytes (PMNs) were obtained from healthy volunteers or patients on chronic HD as indicated. Briefly, whole blood was withdrawn from healthy volunteers, and heparinized samples were placed onto the Mono-poly Resolving Medium (Dainippon Pharmaceutical, Osaka, Japan) to collect PMNs. After centrifugation at 1,800 rpm for 30 min at 18°C, the intermediate PMN-enriched layer was recovered, washed, and resuspended in RPMI 1640 culture medium supplemented with 10% fetal calf serum or phosphate buffered saline (PBS). Collected PMNs were adjusted to a concentration of 5×10^5 cells/ml. 50 μl of cell suspension was placed in a 96-well microplate and 50 μl of the test solution containing methylglyoxal (MGO), a toxic dicarbonyl compound elevated in uremic patients, and/or 4-β phorbol 12-β-myristate 13-α-acetate (PMA; a stimulator of the respiratory burst of PMNs) in PBS, was added. The viability of PMNs was examined using a commercially available kit (CellTiter-Glo® luminescent cell viability assay, Promega Corp., Wisc., USA) as previously reported [17]. After each treatment, 100 μl of the sample was placed in a microplate and 100 μl CellTiter-Glo® reagent (Promega), which contains beetle luciferin and luciferase was added immediately. This reacts with adenosine triphosphate (ATP), which was released from lysed cells, to produce oxyluciferin; it also generates chemiluminescence as a function of the increased ATP levels. After incubation, luminescence was measured using a chemiluminescence analyzer (Glo-Max™ 20/20n luminometer, Promega). Each measurement was made 5 times, and the mean values for each sample were calculated for analysis after correction for cell-free levels.

Measurement of Radical Generation from PMN

The rate of superoxide release from human PMNs was determined by measuring the reduction of ferricytochrome C, as reported elsewhere [18]. PMNs obtained by the same procedure described above were adjusted to a concentration of 1×10^6 cells/ml

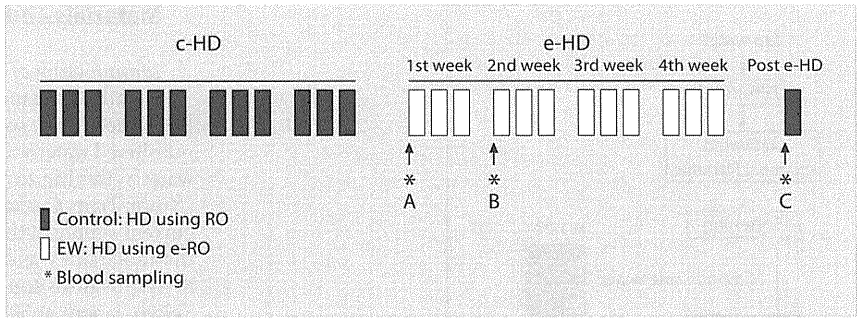

Fig. 2. Feasibility study protocol of HD applying e-RO based HD solution (e-HD). e-RO = reverse osmosis water using electrolyzed water at the cathode side; A = the start of the first e-HD session (first week); B = the start of the fourth e-HD session (second week); C = the start of c-HD after 12 e-HD sessions (post-e-HD).

Table 1. Patient demographics

Case	Sex	Age years	HD vintage months	Underlying renal disease	Dialyser	Prescription	
						AHD	statin
1	female	65	58	NS	BG-1.6	ARB	–
2	male	35	111	CGN	APS-2.1	–	–
3	female	64	216	CGN	BG-1.8	–	–
4	male	70	70	DN	BG-1.8u	ARB	–
5	male	75	77	CGN	AMBC-1.5	ARB	+
6	male	67	335	CGN	APS-185	–	–
7	male	72	116	DN	BG-2.1	CCB+ARB	–
8	male	70	6	NS	PS-1.6	ARB	–

AHD = Anti-hypertensive drug; ARB = angiotensin receptor blockade; CCB = calcium channel blocker; CGN = chronic glomerulonephritis; DN = diabetic nephropathy; NS = nephrosclerosis.

in a solution of 80 μM cytochrome C diluted in PBS. Immediately after adding 10 μl of control or PMA (100 μg) to 700 μl of the sample, 250 μl of the sample was placed on the microplate. Absorbance was measured for 15 min at 550 nm, and was expressed as nanomoles of superoxide production per 0.25 × 10^6 cells/10 min.

In these 2 experiments, PBS was prepared either with control reverse osmosis water (c-RO) or e-RO. Manufactured e-RO was stocked in the closed flask immediately after being made and then used in the study.

Clinical Feasibility of a HD System Using EW

Patients and Study Design

Eight patients on regular HD treatment at Kashima Hospital Dialysis Center (Iwaki, Japan) were enrolled in the trial (table 1). They consisted of 6 men and 2 women, with a mean age of 67 years (range 35–75 years) and a mean dialysis duration of 85 months (range 6–33.5 months). Their underlying renal diseases were chronic glomerulonephritis in 4 patients, nephrosclerosis in 2 and diabetic nephropathy in 2. All patients had been on regular HD treatment 3 times a week for 4 h (n = 6) or 5 h (n = 2) each session. All patients had been using high-flux membrane dialyzers. Among them, 4 were receiving an angiotensin receptor blocker, 1 was receiving an angiotensin receptor blocker and a calcium channel blocker, and 1 was receiving a statin. Patients who were taking ascorbic acid or tocopherol were excluded.

Patients were treated by regular HD regimen using c-HD solution for 1 month, followed by the use of e-HD solution for another month. Both 1-month regimens comprised 3 sessions per week, for a total of 12 sessions (fig. 2). During the study, no changes in HD modes or concomitant medications were made.

Blood sampling was obtained just before each HD session. Blood was immediately centrifuged and serum was stored at –80°C until measurements were made by commercially available kits or high performance liquid chromatography (HPLC). Interleukin-6 was assessed by the CLEIA method (Human IL-6 CLEIA, Fujirebio C, Tokyo, Japan), highly sensitive C reactive protein by nephrometry (N High Sensitive CRP, Dade Behring, Marburg, Germany), creatol and methylguanidine by HPLC, 8-OHdG by an enzyme-linked immunosorbant assay (ELISA) kit (high sensitive 8-OHdG check, Nikken C, Shizuoka, Japan) and pentosidine by an ELISA kit (FSK pentosidine, Fushimi C, Marugame, Japan).

All patients were monitored regarding subjective symptoms during the study periods. Blood pressure was measured using a sphygmomanometer at the upper arm with the patient in the supine position just before and after each HD session. The mean of the 12 measurements obtained was determined.

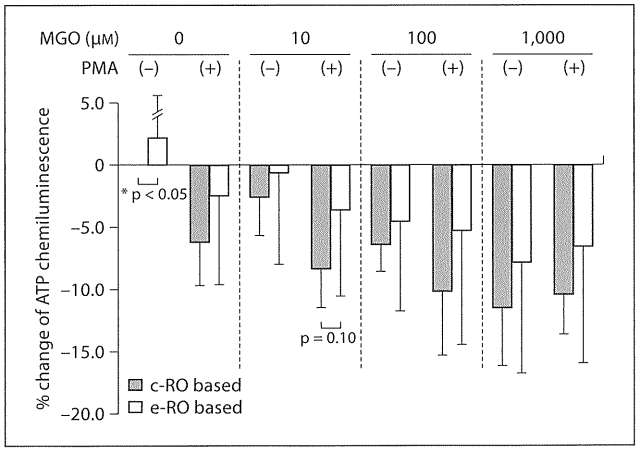

Fig. 3. Effect of e-RO: human neutrophil viability against MGO and/or PMA. Neutrophils from 7 healthy volunteers were suspended in PBS (n = 7). c-RO = treatment by control RO water; e-RO = treatment by RO water by electrolysis. Data are expressed as means ± SD. * $p < 0.05$.

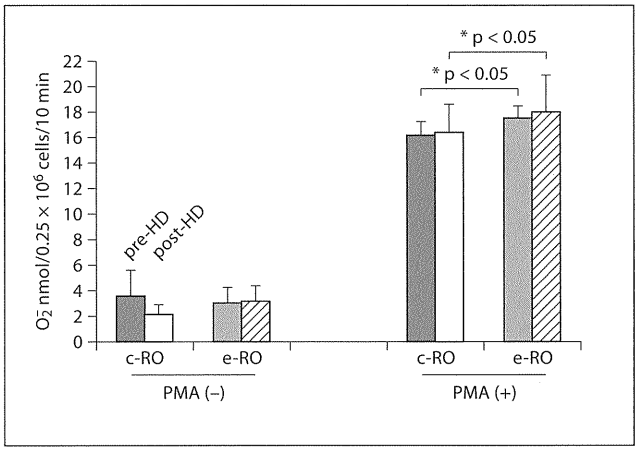

Fig. 4. Effect of e-RO: superoxide anion generation from human neutrophils by PMA. Neutrophils from patients on hemodialysis were suspended in PBS (n = 6). c-RO = treatment by control RO water; e-RO = treatment by RO water by electrolysis. Data are expressed as means ± SD. * $p < 0.05$.

The present study was approved by the ethical committees of Kashima Hospital and Tohoku University (No. 2006-101, No. 2007-207), and informed consent was obtained from all patients. All values are expressed as mean ± SD. Paired t test and 1-way repeated measure analysis of variance were used for statistical analysis. $p < 0.05$ was considered statistically significant.

Results

Changes in ATP-chemiluminescence of PMNs against MGO in the presence or absence of PMA are shown in figure 3. At basal levels (MGO 0 μM) in the absence of PMA, ATP-chemiluminescence was significantly higher with e-RO than c-RO ($p < 0.05$). Significant decreases in ATP-chemiluminescence were associated with MGO levels in both the e-RO and c-RO groups ($p < 0.001$ for both groups). However, no differences were found between the e-RO and c-RO groups in MGO levels. For PMA load, further significant decreases in ATP-chemiluminescence were associated with MGO levels in the e-RO and c-RO groups (at MGO levels 0–100 μM, $p < 0.01$ in both groups); however, no differences were found between c-RO and e-RO.

Figure 4 shows generated superoxide anions in both groups. Under basal levels, no differences were found between groups, whereas in stimulated conditions, levels of generated radicals were significantly higher for e-RO than for c-RO ($p < 0.05$ in pre-HD and post-HD).

Table 2. Change in laboratory parameters

	Pre	Post	p value
WBC, n/μl	6,795 ± 863	6,644 ± 945	0.35
Hb, g/dl	11.3 ± 1.2	11.2 ± 1.2	0.19
Creatinine, mg/dl	11.0 ± 3.2	10.6 ± 3.0	0.07
BUN, mg/dl	64.6 ± 6.2	60.8 ± 9.0	0.1

Data are means ± SD. Blood samples were obtained from the arteriovenous fistula before starting dialysis. Pre = e-HD day 1 (fig. 1); Post = c-HD day 13 (fig. 1); WBC = white blood cells; Hb = hemoglobin; BUN = blood urea nitrogen.

Changes in laboratory parameters during the clinical trial of e-HD are shown in table 2. No changes were observed in white blood cell count, hemoglobin, blood urea nitrogen and creatinine levels. Figure 5 shows changes in inflammatory or oxidative stress markers in the blood. There were significant decreases in plasma methylguanidine levels ($p < 0.05$; fig. 5c). There were also significant decreases in mean blood pressure during the study in the c-HD and e-HD groups between the pre- and post-HD sessions ($p < 0.01$ and $p < 0.001$, respectively; table 3). No adverse symptoms were observed in any patient.

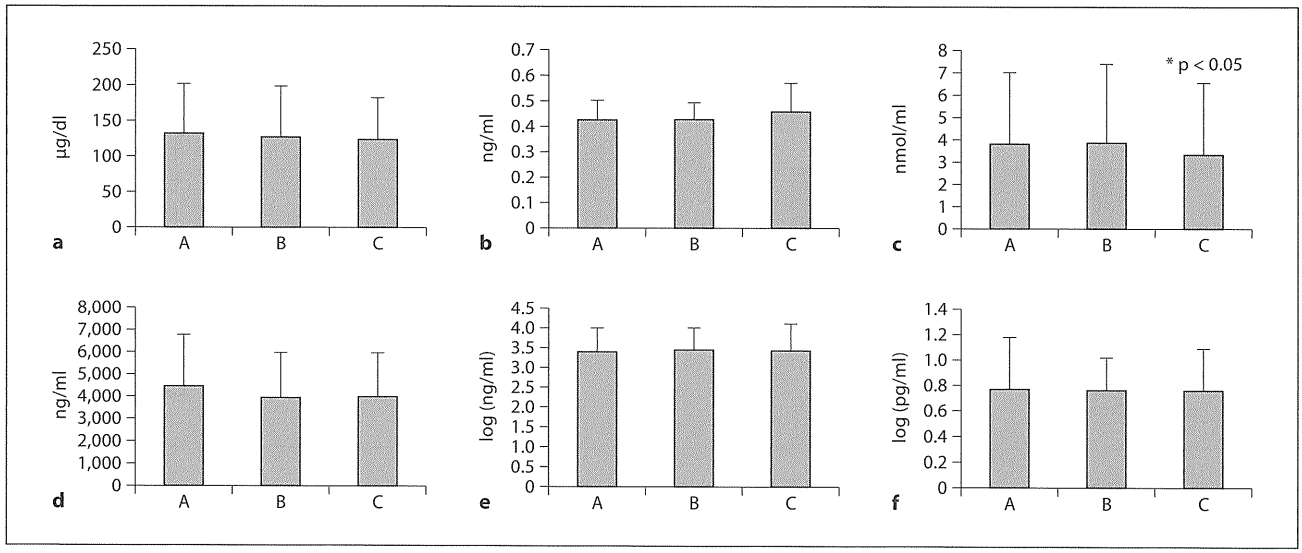

Fig. 5. Changes in laboratory variables after HD using the e-RO based HD solution. **a** Creatol. **b** 8-OHdG. **c** Methylguanidine. **d** Pentosidine. **e** Highly sensitive C reactive protein. **f** Interleukin-6. Data are expressed as means ± SD. A = the start of the first e-HD session (first week); B = the start of the fourth e-HD session (second week); C = the start of c-HD after 12 e-HD sessions (post-e-HD). * $p < 0.05$.

Discussion

It has been demonstrated that e-RO exhibits antioxidative capacity [13–15]. The present study aimed to investigate the biological effect of e-RO on the viability of PMNs, and the clinical feasibility of applying this technology to HD treatment.

Plasma MGO levels are reportedly elevated in patients on dialysis [19, 20]. MGO is highly reactive and biologically toxic due to the formation of advanced glycation end products, protein and DNA modifications [21–23]. Recently, we demonstrated that MGO plays a role in the enhancement of injury to PMNs in combination with H_2O_2 [24], which may be involved with the pathological mechanism of microinflammation in dialysis patients.

In the c-RO group in the present study (fig. 3), decreased viabilities of PMNs were found to be associated with the MGO level, and the toxicity of MGO was further increased in the presence of PMA. Basal cellular viability was better preserved in the e-RO group than in c-RO group. Furthermore, MGO-related PMN injury in the e-RO group was relatively ameliorated compared with the c-RO group, although differences did not reach statistical significance. In addition, in PMNs of uremic patients (fig. 4), the cellular function to generate superoxide anions by a physiological respiratory burst was significantly higher with e-RO than with c-RO.

Table 3. Blood pressure and heart rate pre- and post-dialysis

	c-HD (n = 12)	e-HD (n = 12)	p value
Pre-dialysis			
SBP, mm Hg	165 ± 25	157 ± 21	<0.01
DBP, mm Hg	85 ± 12	81 ± 10	<0.05
MBP, mm Hg	112 ± 15	106 ± 12	<0.01
HR, beats/min	76 ± 12	78 ± 14	0.71
BW gain, %	3.8 ± 0.85	4.0 ± 0.93	0.9
Post-dialysis			
SBP, mm Hg	124 ± 19	117 ± 15	<0.01
DBP, mm Hg	69 ± 11	64 ± 9	<0.001
MBP, mm Hg	87 ± 13	81 ± 11	<0.001
HR, beats/min	80 ± 12	81 ± 14	0.63
Volume removed, kg	2.2 ± 0.8	2.4 ± 0.8	0.97

Data are the mean ± SD levels of 12 HD sessions in each patient. BW gain = Inter-dialytic body weight gain over dry weight; c-HD = hemodialysis using control reverse osmosis water; DBP = diastolic blood pressure; e-HD = hemodialysis using reverse osmosis water by electrolyzed water; HR = heart rate; MBP = mean blood pressure; pre-dialysis = just before the hemodialysis session; post-dialysis = just after the hemodialysis session; SBP = systolic blood pressure; volume removed = intra-dyalitic volume removal per HD session.

These findings indicate that the application of e-RO does not stimulate PMNs, but rather benefits PMNs in preserving cellular viability as compared with c-RO. However, suppression of MGO toxicity by e-RO application was not clearly demonstrated in this study (fig. 3). In terms of the lack of e-RO effect on MGO cytotoxicity in this study (fig. 3), we think that the change in chemical properties of e-RO, such as levels of dissolved hydrogen, may contribute to our findings, as indicated by recent studies that suggested a significant role for dissolved hydrogen in ameliorating oxidative stress [25, 26]. Further studies are needed in this area to address the context of the preservation of e-RO chemical properties.

Next, we examined the feasibility of the application of this technology to regular HD treatment. No symptomatic adverse events were observed during the study period, although there were nonsymptomatic reductions in mean blood pressure before and after the HD session. No changes were found in laboratory parameters or in interdialytic changes of body weight, which may indicate the feasibility of an e-HD system.

In previous reports, Huang et al. [13, 14] showed decreases in surrogate markers of inflammation and oxidative stress following the introduction of an e-HD system. In the present short trial, plasma methylguanidine was significantly decreased, but no other parameters, including C-reactive protein, interleukin-6, creatol and 8-OHdG, were changed (fig. 5). We propose the following reasons for these contradictory results. First, methylguanidine is the nonspecific oxidative end product that could reflect the overall oxidative milieu, and thus could be changed significantly. Second, we only enrolled clinically stable patients, and thus those who presented with inflammation or malnutrition were not included in the study. This clinical background might influence the results of our study.

Interestingly, MBP was decreased by the introduction of e-HD. Taken together, the findings of decreased MBP and no changes in heart rate or inter-dialytic body weight gain lead us to speculate that e-HD may lead to vasodilation. Vasoconstrictive action by radical oxygen species such as peroxynitrite could play a role in increased blood pressure in HD patients, and dissolved hydrogen in EW may reduce superoxide anions, as previously reported [8], during the course of e-HD to suppress its pathological process. Further studies are needed in this area.

There are some unresolved issues in the present HD system. Data on the microbiological quality of the dialysis solution were not available, and water volume consumed per session was not clearly measured. These issues need to be clarified in follow-up studies.

In conclusion, the present study demonstrated the capacity of e-RO to preserve the viability of PMNs, and the clinical feasibility of using this water for HD treatment. The clinical application of this technology may improve bio-compatibility of HD treatment.

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Modification of the CKD Epidemiology Collaboration (CKD-EPI) Equation for Japanese: Accuracy and Use for Population Estimates

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Introduction: We previously reported a modification to the Modification of Diet in Renal Disease (MDRD) Study equation for use in Japan. Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) developed a new equation that is more accurate and yields a lower CKD prevalence estimate in the United States than the MDRD Study equation. We modified the CKD-EPI equation for use in Japan, compared its accuracy with the Japanese modification of the MDRD Study equation, and compared the prevalence of CKD in Japan using both equations.

Design: A diagnostic test study comparing the Japanese coefficient–modified CKD-EPI equation and Japanese coefficient–modified MDRD Study equation and a cross-sectional study comparing distribution of estimated glomerular filtration rate and prevalence of CKD in participants in a Japanese annual health check program.

Setting & Participants: 763 Japanese patients (413 for development and 350 for validation) were included. Prevalence estimates were based on 574,024 participants from the annual health check program.

Index Test: Japanese modification of the MDRD Study and CKD-EPI equations.

Reference Test: Inulin clearance.

Results: The Japanese coefficient of the modified CKD-EPI equation was 0.813 (95% CI, 0.794-0.833). In the validation data set, the modified CKD-EPI equation performed better than the modified MDRD Study equation. Bias (measured GFR [mGFR] − eGFR) was 0.4 ± 17.8 (SD) versus 1.3 ± 19.8 mL/min/1.73 m^2 overall, respectively ($P = 0.02$); 7.3 ± 20.6 versus 7.8 ± 22.2 mL/min/1.73 m^2 for participants with mGFR ≥60 mL/min/1.73 m^2, respectively ($P < 0.001$); and −4.4 ± 13.8 versus −3.3 ± 15.6 mL/min/1.73 m^2 for participants with mGFR <60 mL/min/1.73 m^2, respectively ($P = 0.5$). The modified CKD-EPI equation yields a lower estimated prevalence of CKD than the modified MDRD Study equation (7.9% vs 10.0%), primarily because of a lower estimated prevalence of stage 3 (5.2% vs 7.5%).

Limitation: Most study participants had CKD. The study population contained a limited number of participants with mGFR ≥90 mL/min/1.73 m^2.

Conclusion: The Japanese coefficient–modified CKD-EPI equation is more accurate than the Japanese coefficient–modified MDRD Study equation and leads to a lower estimated prevalence of CKD in Japan.

Am J Kidney Dis 56:32-38.

INDEX WORDS: Modification of Diet in Renal Disease (MDRD) Study equation; Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation; CKD prevalence; Japanese coefficient.

Accurate estimation of glomerular filtration rate (GFR) is crucial for the detection of chronic kidney disease (CKD).[1] Calculating GFR by measuring the clearance of exogenous markers, such as inulin, is accurate, but the procedure is time consuming. The use of GFR-estimating equations has been recommended in clinical practice.[1] The Modification of Diet in Renal Disease (MDRD) Study equation[2] is the most commonly used worldwide. The equation was developed in mostly whites and African Americans. We previously reported that estimated GFR (eGFR) obtained using the isotope-dilution mass spectrometry–traceable 4-variable MDRD Study equation was significantly higher than measured GFR (mGFR) in Japanese patients.[3] Therefore, we calculated a correction coefficient of 0.808 for the MDRD

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Study equation and developed a new Japanese equation for GFR estimation.[3]

Recently, Levey et al[4] developed a more accurate new GFR estimation equation, the CKD Epidemiology Collaboration (CKD-EPI) equation, based on data from 5,504 participants. The equation yields a lower estimated prevalence of CKD than the MDRD Study equation in the United States. In this study, we explored the accuracy of this new equation in Japanese and estimated CKD prevalence in the general population in Japan using the equation. Because the CKD-EPI equation was developed mostly in whites and African Americans, we calculated a correction coefficient for the use of the CKD-EPI equation in Japanese and performed: (1) a diagnostic test study comparing the Japanese coefficient–modified CKD-EPI equation with the Japanese coefficient–modified MDRD Study equation, and (2) cross-sectional study comparing the distribution of eGFR and prevalence of CKD in participants in a Japanese annual health check program.

METHODS

Diagnostic Test Study

Participants

To perform a diagnostic test study to compare the modified CKD-EPI and modified MDRD Study equations, we used same data sets from which the Japanese coefficient of the MDRD Study equation was developed and validated. Details of participants were reported previously.[3] Briefly, 763 Japanese patients in 80 medical centers were included. They were divided into a development data set (413 participants) and a validation data set (350 participants). GFR was measured using inulin renal clearance. Serum creatinine was measured using an enzymatic method in a single laboratory. The accuracy of creatinine measurement was validated using the calibration panel of the Cleveland Clinic.[3]

Calculation of a Coefficient of the CKD-EPI Equation

A coefficient for the CKD-EPI equation appropriate for use in Japanese was calculated in the development data set in the same way the Japanese MDRD Study equation coefficient was obtained previously.[3] The coefficient was determined by minimizing the sum of squared errors between eGFR and inulin renal clearance.

Performance of the Coefficient-Modified Equation

Performance of the Japanese coefficient–modified equations was studied using the development and validation data sets. Bias, root mean square error, and accuracy within 30% (P_{30}) were analyzed.

Cross-sectional Study

Population

We previously reported the prevalence of CKD based on data from the Japanese annual health check program in 2005 using an equation for Japanese.[5] In the present study, to compare eGFR distribution and CKD prevalence in participants in this health check program, we used the same population from the Japanese annual health check program, which consisted of 574,024 participants older than 20 years. Details of the data have been reported previously.[5] We calculated CKD prevalence using the Japanese coefficient–modified MDRD Study equation and Japanese coefficient–modified CKD-EPI equation using a Japanese adult population obtained from a census in 2005.

Statistical Analysis

Data are expressed as mean ± standard deviation. Differences in clinical characteristics between the development and validation

Table 1. Clinical Characteristics of the Study Population for the Diagnostic Test Study

Characteristic	Development Data Set	Validation Data Set	*P*
No. of participants	413	350	
Men	262 (63)	203 (58)	0.1
Age (y)	51.4 ± 16.5	53.9 ± 17.5	0.04
Height (cm)	163.2 ± 8.8	161.6 ± 9.5	0.01
Weight (kg)	61.0 ± 12.9	60.4 ± 12.7	0.5
BSA (m^2)	1.65 ± 0.19	1.63 ± 0.19	0.2
BMI (kg/m^2)	22.8 ± 3.8	23.0 ± 3.8	0.4
Diabetes	82 (20)	77 (22)	0.5
Hypertension	235 (57)	202 (58)	0.8
Transplant	9 (2)	2 (1)	0.06
Kidney donor	1 (0)	10 (3)	0.003
Creatinine (mg/dL)	1.52 ± 1.59	1.88 ± 1.70	0.6
mGFR (mL/min/1.73 m^2)	59.1 ± 35.4	45 ± 25	0.5

Note: Data are expressed as mean ± standard deviation or number (percentage). Conversion factor for GFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667.

Abbreviations: BMI, body mass index; BSA, body surface area; mGFR, measured glomerular filtration rate.

Table 2. Performance of GFR-Estimating Equations in the Validation Data Set

Variable and Equation	All (N = 350)	mGFR <60 mL/min/1.73 m^2 (n = 206)	mGFR ≥60 mL/min/1.73 m^2 (n = 144)
Bias (mL/min/1.73 m^2)			
Japanese coefficient–modified MDRD Study equation	1.3 ± 19.4	−3.3 ± 15.6	7.8 ± 22.2
Japanese coefficient–modified CKD-EPI Study equation	0.4 ± 17.8	−4.4 ± 13.8	7.3 ± 20.6
P	0.02	0.5	<0.001
P_{30} (%)			
Japanese coefficient–modified MDRD Study equation	73 (69-78)	67 (61-74)	82 (75-87)
Japanese coefficient–modified CKD-EPI Study equation	75 (70-79)	65 (58-71)	88 (82-92)
P	0.7	0.6	0.1
Root mean square error (mL/min/1.73 m^2)			
Japanese coefficient–modified MDRD Study equation	19.4	15.9	23.5
Japanese coefficient–modified CKD-EPI Study equation	17.8	14.4	21.8

Note: Bias is mGFR minus eGFR and is reported as mean ± standard deviation; P_{30} refers to percentage of GFR estimates that are within 30% of mGFR, with 95% confidence intervals given in parentheses. The Japanese coefficient–modified MDRD Study equation is the isotope-dilution mass spectrometry–traceable 4-variable MDRD Study equation multiplied by a Japanese coefficient of 0.808: $eGFR = 0.808 \times 175 \times SCr^{-1.154} \times Age^{-0.203} \times 0.742$ (if female). The Japanese coefficient–modified CKD-EPI Study equation is multiplied by a Japanese coefficient of 0.813; $eGFR = 0.813 \times 141 \times \min(SCr/\kappa, 1)^{\alpha} \times \max(SCr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if black], where SCr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1.

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; mGFR, measured glomerular filtration rate.

data sets were evaluated using χ^2 test and independent *t* test. Differences in the bias (absolute value) of eGFRs were evaluated using paired *t* test. Differences in accuracy (ie, P_{30}) were evaluated using χ^2 tests. Differences in the prevalence of specific GFR groups were evaluated using χ^2 test. A difference with $P < 0.05$ is considered statistically significant. Statview, version 4.02, and JMP 8.01 (both from SAS Institute, www.sas.com) were used for statistical analysis. JMP 8.01 was used for receiver operating characteristic curve analysis.

RESULTS

Modifying the CKD-EPI Equation for a Japanese Population

The coefficient to modify the CKD-EPI equation for Japanese, calculated from the development data set of 413 participants (for whom clinical characteristics are listed in Table 1), was found to be 0.813 (95% confidence interval, 0.794-0.833).

Diagnostic Test Study

We used a diagnostic test design to compare the Japanese coefficient–modified CKD-EPI and MDRD Study equations, which are listed in Table 2.

Comparison of Performance of Coefficient-Modified Equations

We analyzed all participants and subgroups in the validation data set, stratified by mGFR (<60 vs ≥60 mL/min/1.73 m^2; Table 2). As in the development data set, root mean square error was lower for the Japanese coefficient–modified CKD-EPI equation than the Japanese coefficient–modified MDRD Study equation in all participants and both subgroups stratified by mGFR. The coefficient-modified CKD-EPI equation had significantly less bias than the coefficient-modified MDRD Study equation in all participants ($P = 0.02$). This difference was due to improved bias in participants with GFR ≥60 mL/min/1.73 m^2 ($P < 0.001$); there was no significant difference in bias in participants with GFR <60 mL/min/1.73 m^2. Accuracy was not significantly different between equations.

Table 3 lists the performance of the equations in a validation data set (see Table 1 for details of participants in this data set) stratified by clinical characteristics. Compared with the coefficient-modified MDRD Study equation, the coefficient-modified CKD-EPI equation showed significantly lower bias in younger participants (aged

Table 3. Performance of Japanese Coefficient–Modified GFR-Estimating Equations in the Validation Data Set According to Clinical Characteristics

Clinical Characteristics	No. of Participants	Bias		*P*
		0.808 × MDRD	0.813 × CKD-EPI	
Sex				
Men	203	0.8 ± 15.8	0.4 ± 14.7	0.1
Women	147	1.9 ± 23.4	0.5 ± 21.5	0.1
Age (y)				
19-44	107	3.2 ± 18.7	−0.5 ± 17.1	0.03
45-64	130	1.0 ± 22.5	1.1 ± 20.7	0.5
≥65	113	−0.2 ± 15.9	0.5 ± 14.7	0.1
BMI (kg/m^2)				
<20	71	0.2 ± 26.4	−0.5 ± 25	0.9
20-25	190	−0.6 ± 17.2	−1.2 ± 14.8	0.01
>25	89	6.1 ± 16.2	4.6 ± 16.4	0.2
Diabetes				
Yes	83	−1.5 ± 15.2	−1.1 ± 14.5	0.9
No	264	2.2 ± 20.5	0.9 ± 18.8	0.02
Hypertension				
Yes	209	1.0 ± 15.9	0.1 ± 15.5	0.7
No	141	1.6 ± 23.6	0.9 ± 20.9	0.02
Total	350	1.3 ± 19.4	0.4 ± 17.8	0.02

Note: Unit of bias (mGFR − eGFR) is mL/min/1.73 m^2. Bias was reported as mean ± standard deviation. 0.808 × MDRD refers to the Japanese coefficient–modified isotope-dilution mass spectrometry–traceable 4-variable MDRD Study equation. 0.813 × CKD-EPI refers to the Japanese coefficient–modified CKD-EPI Study equation.

Abbreviations: BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; mGFR, measured glomerular filtration rate.

19-44 years; $P = 0.03$), those with optimal body mass index (20-25 kg/m^2; $P = 0.01$), those without diabetes ($P = 0.02$), and those without hypertension ($P = 0.02$).

Receiver operating characteristic curves to detect GFRs less than 90, 60, and 30 mL/min/1.73 m^2 did not differ between the Japanese coefficient–modified CKD-EPI and MDRD Study

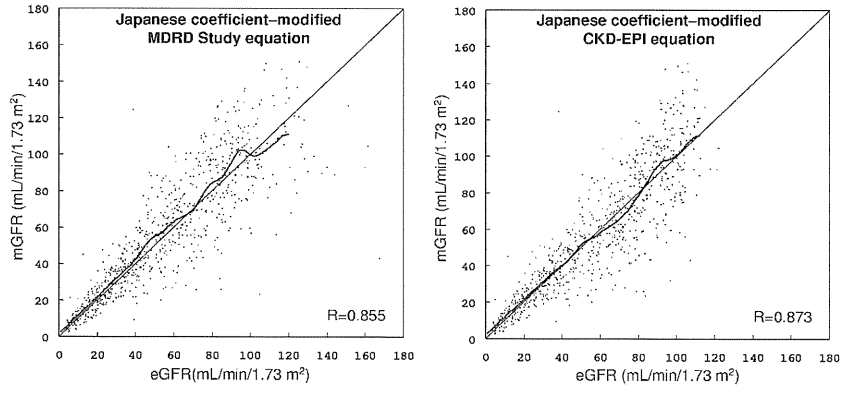

Figure 1. Correlation between estimated (eGFR) and measured glomerular filtration rate (mGFR) in the combined data set. (Left) mGFR versus eGFR obtained using the Japanese coefficient–modified Modification of Diet in Renal Disease (MDRD) Study equation. (Right) mGFR versus eGFR obtained using the Japanese coefficient–modified Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Smoothed lines show the fit of the data.

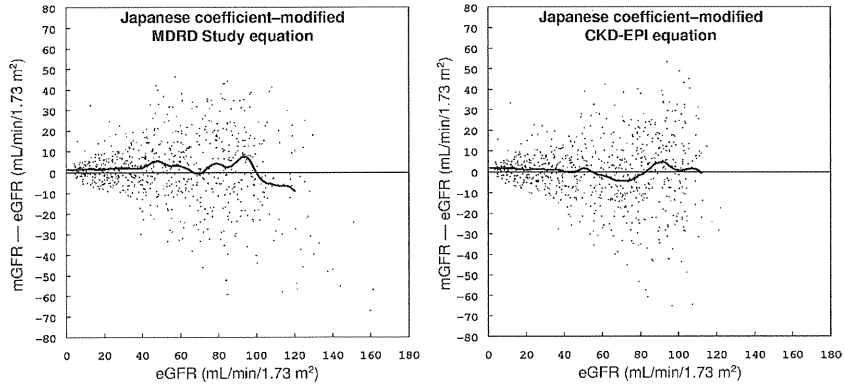

Figure 2. Difference between measured (mGFR) and estimated glomerular filtration rate (eGFR) versus eGFR in the combined data set. (Left) mGFR minus eGFR versus eGFR obtained using the Japanese coefficient–modified Modification of Diet in Renal Disease (MDRD) Study equation. (Right) mGFR minus eGFR versus eGFR obtained using the Japanese coefficient–modified Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

equations. Areas under the receiver operating characteristic curves were 0.93, 0.94 and 0.96 for both equations, respectively.

Correlation Between Modified CKD-EPI eGFR and mGFR

The correlation coefficient between mGFR and eGFR calculated using the coefficient-modified CKD-EPI equation in the combined data set was higher than the corresponding value for the coefficient-modified MDRD Study equation (0.872 vs 0.855, respectively; Fig 1). Smoothed lines show the fit of the data. Plots of mGFR minus eGFR versus eGFR were evaluated as shown in Fig 2. Smoothed lines show the fit of the data. The Japanese coefficient–modified CKD-EPI equation showed good performance.

Cross-sectional Study

We also performed a cross-sectional study to compare the eGFR distribution and CKD prevalence obtained using the Japanese coefficient–modified equations in participants in a Japanese annual health check program. Characteristics of the study population are shown in Table 4 and results of the cross-sectional analysis are shown in Fig 3. Percentages of specific GFR ranges (15-29, 30-59, 60-89, 90-119, and ≥120 mL/min/1.73 m^2) indicated that the coefficient-modified CKD-EPI equation increased the prevalence of GFR within the range of 90-119 mL/min/1.73 m^2 from 28.6% to 34.0% and decreased the prevalence of GFR within the range of 30-59 mL/min/1.73 m^2 from 7.5% to 5.2%. The coefficient-modified CKD-EPI equation yields a lower estimated prevalence of CKD than the coefficient-modified MDRD Study equation (7.9% vs 10.0%), primarily because of a lower estimated prevalence of stage 3 (5.2% vs 7.5%).

Table 4. Characteristics of the Study Population in the Annual Health Check Program

	Men	Women
No. of participants	240,594	333,430
Age (y)	57.8	58.6
Creatinine (mg/dL)	0.86	0.63
Mean eGFR (mL/min/1.73 m^2)		
0.808 × MDRD	78.5	81.9
0.813 × CKD-EPI	77.5	79.6
Median eGFR (mL/min/1.73 m^2)		
0.808 × MDRD	77 (68-88)	79 (70-93)
0.813 × CKD-EPI	78 (70-86)	80 (73-87)
Prevalence (%)		
Diabetes	5.9	3.5
Hypertension	30.3	24.7
Proteinuria	4.7	2.5

Note: Values in parentheses are interquartile ranges. 0.808 × MDRD refers to the Japanese coefficient–modified isotope-dilution mass spectrometry–traceable 4-variable MDRD Study equation. 0.813 × CKD-EPI refers to the Japanese coefficient–modified CKD-EPI Study equation.

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease.

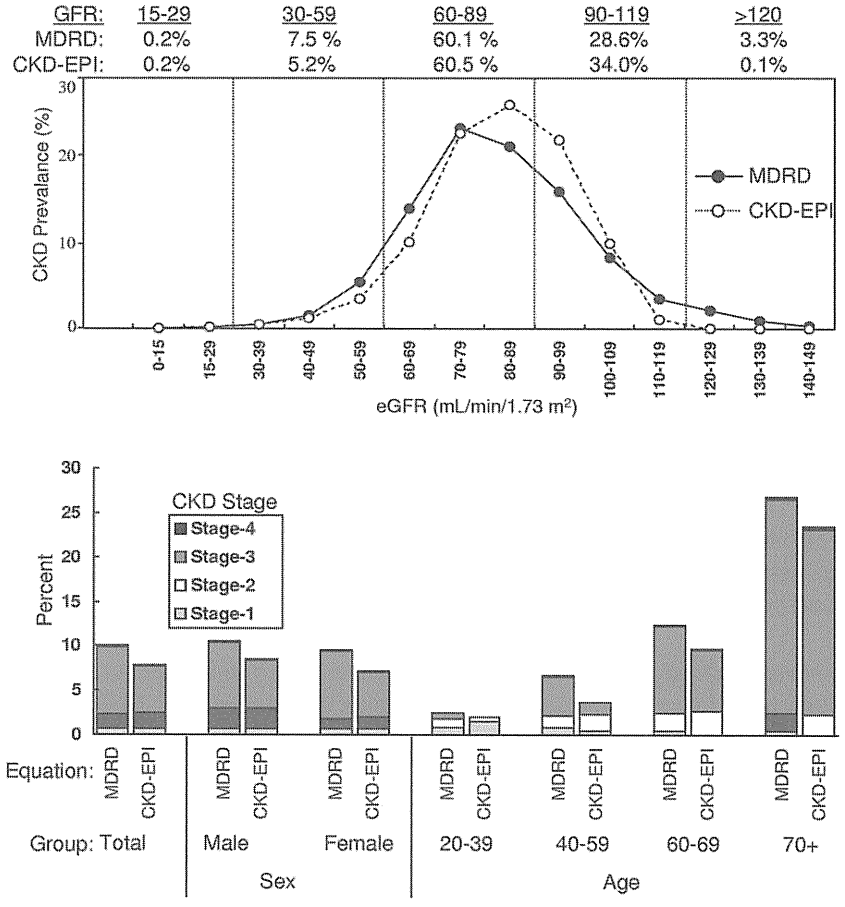

Figure 3. Comparison of distributions of estimated glomerular filtration rate (eGFR) and chronic kidney disease (CKD) prevalence. (Top) Distribution in a Japanese general adult population of eGFR obtained using the Japanese coefficient–modified Modification of Diet in Renal Disease (MDRD) Study equation (solid line) compared with eGFR obtained using the Japanese coefficient–modified Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (dotted line). Percentages of specific GFR ranges (15-29, 30-59, 60-89, 90-119, and ≥120 mL/min/1.73 m^2) are shown. (Bottom) Estimated prevalence of CKD by sex and age when GFRs are obtained using either the Japanese coefficient–modified MDRD Study or CKD-EPI equation.

DISCUSSION

We previously reported a Japanese coefficient of 0.808 for the MDRD Study equation.[3] In the present study, we obtained the Japanese coefficient of 0.813 (95% confidence interval, 0.794-0.833) for the CKD-EPI equation. The values are similar in both equations. The observation that correction coefficients are less than 1.0 indicates lower serum creatinine levels in Japanese than in whites with equivalent GFRs, probably because of the lower skeletal muscle mass found in Japanese compared with North Americans.[3]

The coefficient-modified CKD-EPI equation had lower bias (P = 0.02) than the coefficient-modified MDRD Study equation because of lower bias in participants with mGFR ≥60 mL/min/1.73 m^2. As reported by Levey et al,[4] the improvement in bias likely depends on the use of a 2-slope linear spline with sex-specific knots to model the relationship between log(GFR) and log(serum creatinine), which allows for a steeper slope of GFR versus serum creatinine at creatinine levels above the knots and a less steep slope at creatinine levels below the knots.[4] Differences in bias between subgroups defined by age, body mass index, diabetes, and hypertension also were noted, but larger studies are needed to confirm these results.

The eGFR distribution and CKD prevalence indicated that the Japanese coefficient–modified CKD-EPI equation increased the prevalence of GFR within the range of 90-119 mL/min/1.73 m^2 even as it decreased the prevalence of GFR

within the range of 30-59 mL/min/1.73 m^2. The coefficient-modified CKD-EPI equation yields a lower estimated prevalence of CKD than the coefficient-modified MDRD Study equation (7.9% vs 10.0%), primarily because of a lower estimated prevalence of stage 3 (5.2% vs 7.5%). This result may be explainable by the characteristics of the coefficient-modified CKD-EPI equation that increased eGFR in participants stratified by mGFR >60 or <60 mL/min/1.73 m^2 compared with the coefficient-modified MDRD Study equation. Levey et al[4] reported that the CKD-EPI equation decreased the prevalence estimate for CKD in the United States from 13.1% to 11.5% compared with the MDRD Study equation. These results are consistent with our results.

Limitations of the present study are as follows. (1) We obtained and validated the Japanese coefficient for the CKD-EPI equation from 763 participants. Most study participants had CKD. The study population contained a limited number of participants with mGFR ≥90 mL/min/1.73 m^2, and performance of the coefficient-modified equation was not studied sufficiently in the healthy population. (2) We compared performances between coefficient-modified equations, but the best performance of the equations may not be shown by a simple coefficient correction. The CKD-EPI equation uses log(serum creatinine) with 2-slope linear spline with sex-specific knots at 0.7 mg/dL in women and 0.9 mg/dL in men. That the coefficient was found to be less than 1.0 indicates lower serum creatinine levels in Japanese than in whites with equivalent GFRs. It is unknown whether creatinine values for sex-specific knots are suitable for Japanese.

In conclusion, the CKD-EPI equation modified with the Japanese coefficient performed better than the Japanese coefficient–modified MDRD Study equation. The Japanese coefficient–modified CKD-EPI equation yields a lower estimated prevalence of CKD than the Japanese coefficient–modified MDRD Study equation, primarily because of a lower estimated prevalence of CKD stage 3.

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