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ORIGINAL ARTICLE

Effects of smoking and its cessation on creatinine- and cystatin C-based estimated glomerular filtration rates and albuminuria in male patients with type 2 diabetes mellitus: the Fukuoka Diabetes Registry

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Cigarette smoking is an important modifiable risk factor for lifestyle diseases. The smoking rate remains high, and the prevalence of diabetes mellitus is increasing in Asian countries; however, few studies have examined the effects of smoking on chronic kidney disease (CKD) in Asian diabetic patients. The aim of the present study was to investigate the association between smoking and its cessation with CKD and its components in patients with type 2 diabetes. A total of 2770 Japanese male patients with type 2 diabetes aged ⩾20 years were divided according to the amount of cigarette smoking and the years since cessation. The associations with CKD, the urinary albumin–creatinine ratio (UACR) and the estimated glomerular filtration rate (eGFR) were cross-sectionally examined. The proportions of CKD and the mean UACR dose-dependently increased with increases in both the number of cigarettes per day and the Brinkman index compared with the never smokers. The creatinine-based eGFR also increased with increases in the amount of smoking, whereas the cystatin C-based eGFR decreased, and their average did not significantly change. These parameters exhibited inverse associations with the years after smoking cessation compared with the association with the amount of smoking. A dose-dependent association of active smoking and a graded inverse association of the years since quitting with CKD enhance the merit of smoking cessation in patients with type 2 diabetes.
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Keywords: albuminuria; chronic kidney diseases; diabetes mellitus; smoking; smoking cessation

INTRODUCTION

Cigarette smoking has been established as a modifiable risk factor for lifestyle diseases because of its adverse consequences, such as type 2 diabetes mellitus,[1] cardiovascular disease[2,3] and premature death.[4] The prevalence of smoking has decreased; however, smokers tend to lose at least 10 years of life compared with individuals who have never smoked, even in the twenty-first century.[4] Chronic kidney disease (CKD) has been recognized as a strong predictor for end-stage renal failure and cardiovascular disease.[5] Regarding the associations with smoking, an increased risk of CKD has been reported;[6–9] however, the definition of CKD has differed across studies. When examining the components of CKD separately, most studies have demonstrated a positive association between smoking and proteinuria.[6,8,10–16] In contrast, an association with renal function has yielded inconsistent results, including an increased[6,10,12–14] or decreased[7,8,13,15–17] relationship in cross-sectional[6,7,10,12,14,15,17] and longitudinal[7,8,12,13,16,17] studies. Furthermore, to date, in contrast to active smoking, the relationships among smoking cessation and CKD-associated factors have not been fully examined.

Despite the previously described adverse effects of smoking, the smoking rate remains high in Asian countries.[18] Furthermore, the prevalence of diabetes is rapidly increasing in Asia.[19,20] Therefore, it is helpful to clarify the association between smoking and CKD among Asian diabetic patients for the effective prevention of diabetic complications.

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Direct measurement of the glomerular filtration rate (GFR) is impractical; thus, equations that estimate the GFR have been developed using serum concentrations of endogenous filtration markers. Following the standardization of serum creatinine and cystatin C measurements,[21,22] the Chronic Kidney Disease Epidemiology Collaboration developed equations based on standardized creatinine in 2009[23] and standardized cystatin C in 2012.[24] However, there are racial differences in the Chronic Kidney Disease Epidemiology Collaboration equations for the estimated GFR (eGFR); thus, the Japanese Society of Nephrology developed creatinine- and cystatin C-based eGFRs for Japanese subjects in 2009[25] and 2013,[26] respectively. The Japanese eGFR equations perform well compared with the coefficient-modified Chronic Kidney Disease Epidemiology Collaboration equations[27] and may be used for other Asian populations.[28] The association between smoking and CKD has been investigated using serum creatinine in most previous studies; thus, it may be meaningful to classify CKD with the eGFR calculated by alternative equations because of the potential effects of non-renal factors on serum creatinine.[26] In this context, the aim of the present study was to examine the associations between smoking and its cessation with the prevalence of CKD, creatinine- and cystatin C-based eGFRs and urinary albumin excretion in patients with type 2 diabetes.

METHODS

Study participants

The Fukuoka Diabetes Registry is a multicenter prospective study designed to investigate the influence of modern treatments on the prognoses of patients with diabetes who regularly attend teaching hospitals certified by the Japan Diabetes Society or certified diabetes clinics in Fukuoka Prefecture, Japan (UMIN Clinical Trial Registry 000002627).[29] A total of 5131 patients with diabetes aged 20 years or older were registered between April 2008 and October 2010. The exclusion criteria of the registry were as follows: (1) patients with drug-induced diabetes or undergoing steroid treatment; (2) patients undergoing renal replacement therapy; (3) patients with serious diseases other than diabetes, such as advanced malignancies or decompensated liver cirrhosis; and (4) patients unable to regularly visit diabetologists. After excluding 261 subjects with type 1 diabetes, 2095 female subjects, four subjects for whom the smoking duration was unavailable and one subject without a cystatin C measurement, the remaining 2770 subjects were included in this cross-sectional analysis. This study was conducted with the approval of the Kyushu University Institutional Review Board, and written informed consent was obtained from all participants.

Clinical evaluation

The participants completed a self-administered questionnaire to collect information regarding smoking habits, duration of diabetes, alcohol intake, physical activity, diet and depressive symptoms. Based on the smoking status, the patients were classified as never smokers, past smokers or current smokers in accordance with our previous report.[30] Never smokers were defined as individuals who had never smoked. Past smokers were defined as individuals who smoked before but did not smoke at the time of study registration. Current smokers were further subdivided by the number of cigarettes per day (<20, 20–29 or $\geqslant 30$ cigarettes per day) and the Brinkman index (calculated by the number of cigarettes per day multiplied by the number of years smoked, <600, 600–999 or $\geqslant 1000$). Past smokers were subclassified according to the years since quitting (<10, 10–19 or $\geqslant 20$ years). Alcohol intake was classified as the presence or absence of current use. The participants who regularly engaged in sports during their leisure time were defined as the regular exercise group. A dietary survey was conducted using a brief-type, self-administered, diet history questionnaire regarding the food frequency of 58 items (BDHQ; Gender Medical Research, Tokyo, Japan). The validity of ranking the energy-adjusted intakes of many nutrients has been previously investigated in an adult Japanese population.[31] The presence of depressive symptoms was assessed using the Center for Epidemiologic Studies Depression Scale;[32] the participants who scored 16 or more out of 60 points were defined as having depressive symptoms. The body mass index (BMI) was calculated from the height and weight. The blood pressure was measured with the participant in a sitting position. Medication use was determined based on medical records. The participants were categorized by the use or lack of oral hypoglycemic agents, insulin therapy and renin–angiotensin system inhibitors. Hypertension was defined as a blood pressure $\geqslant 140/90$ mm Hg and/or current use of antihypertensive agents.

Laboratory measurements

Blood was collected via venipuncture. Hemoglobin A_{1c} (HbA_{1c}) was determined using high-performance liquid chromatography (Tosoh Corp., Tokyo, Japan). Serum cystatin C was determined via latex immunonephelometry (Iatoro Cys-C; LSI Medience, Tokyo, Japan) and standardized to ERM-DA471/IFCC using the transfer factor proposed by the Japan Society of Clinical Chemistry: IFCC standard cystatin $C = 1.09 \times$ cystatin C.[33] The creatinine-based eGFR ($eGFR_{cr}$) and the cystatin C-based eGFR ($eGFR_{cys}$) were calculated using the equations proposed by the Japanese Society of Nephrology[25,26] as follows:

$$eGFR_{cr}\left(\mathrm{ml\,min^{-1}\ per\ 1.73\,m^2}\right) = 194 \times Cr^{-1.094} \times age^{-0.287} \times 0.739\ (\text{if female})$$

$$eGFR_{cys}\left(\mathrm{ml\,min^{-1}\ per\ 1.73\,m^2}\right) = \left[104 \times CysC^{-1.019} \times 0.996^{age} \times 0.929\ (\text{if female})\right] - 8$$

The average eGFR ($eGFR_{average}$) comprised the mean of the creatinine- and cystatin C-based eGFRs.[26] A spot urine sample was obtained, and the urinary creatinine and albumin were measured using an enzymatic method and an immunonephelometry method (Medical and Biological Laboratories, Nagoya, Japan), respectively. The urinary albumin–creatinine ratio (UACR) ($mg\,g^{-1}$) levels were calculated by dividing the urinary albumin by the urinary creatinine concentrations. Albuminuria was defined as a urinary albumin excretion $\geqslant 30\ mg\,g^{-1}$ creatinine. CKD was defined as albuminuria and/or an eGFR $<60\ ml\,min^{-1}$ per 1.73 m^2.[5]

Statistical analysis

Differences in the mean values or proportions of the characteristics of the studied participants were tested using an unpaired *t*-test, analysis of variance or chi-square test, as appropriate. The age-adjusted mean values for the UACR and eGFR were calculated via an analysis of covariance. The UACR was log-transformed for statistical analyses because of a skewed distribution, back-transformed and reported with the 95% confidence intervals (CIs). The multivariate-adjusted partial regression coefficients and their 95% CIs of the log-transformed UACR and eGFR were determined using multiple regression analysis and were examined for linear trends using multiple regression analysis. The proportions of the patients with CKD were adjusted for age by a direct method using all study subjects as a standard population and were compared and examined for trends via logistic regression analysis. All analyses were performed using the SAS software package version 9.3 (SAS Institute, Cary, NC, USA). Values of $P<0.05$ were considered statistically significant in all analyses.

RESULTS

Table 1 indicates the clinical characteristics of the study participants according to the smoking status. The mean age and duration of diabetes tended to decrease in the current smokers compared with the never smokers and past smokers. Regarding the smoking-related parameters, the duration of smoking was longer in the current smokers compared with the past smokers, whereas the number of cigarettes per day and the Brinkman index were greater in the past smokers. The total protein intake and the proportion of individuals with regular exercise habits were likely to decrease, whereas the individuals with a current drinking habit and depressive symptoms increased in the current smokers. The HbA_{1c} level and the frequency of patients on insulin therapy increased in the current smokers. The proportion of patients using renin–angiotensin system inhibitors

Table 1 Clinical characteristics of the study subjects according to smoking status

	Never smoker	*Past smoker*	*Current smoker*	P-*value*
N	559	1451	760	
Age (years)	65.7±10.6	66.9±9.2	61.3±9.9	<0.001
Duration of diabetes (years)	17.3±11.0	17.3±11.1	14.7±10.3	<0.001
Duration of smoking (years)		29.7±13.8	41.6±10.1	<0.001
Years since quitting (years)		17.6±13.4		
Number of cigarettes per day		30.6±20.9	21.3±11.8	<0.001
Brinkman index		943±778	874±519	0.03
Total energy intake (kcal per day)	1834±478	1831±513	1809±518	0.56
Total protein intake (g per day)	71.2±22.7	71.9±26.2	69.0±24.5	0.04
Current drinker (%)	50	57	60	0.001
Regular exercise (%)	73	78	63	<0.001
Depressive symptoms (%)	7	6	10	0.004
BMI (kg m^{-2})	23.4±3.3	23.6±3.0	23.7±3.6	0.25
HbA_{1c} (%)	7.28±0.94	7.28±0.96	7.53±1.14	<0.001
Oral hypoglycemic agents use (%)	64	63	63	0.90
Insulin use (%)	23	26	31	0.01
Systolic blood pressure (mm Hg)	130±16	131±17	129±16	0.02
Diastolic blood pressure (mm Hg)	76±10	75±11	76±10	0.30
RAS inhibitors use (%)	42	47	42	0.03
Hypertension (%)	60	65	59	0.007
Cr (mg dl^{-1})	0.94±0.55	0.93±0.42	0.85±0.45	<0.001
Cystatin C (mg l^{-1})	0.98±0.44	0.98±0.36	0.96±0.38	0.47
UACR (mg g^{-1})	16.3 (7.2–57.8)	19.7 (7.8–80.1)	21.2 (8.4–80.8)	0.01
$eGFR_{cr}$ (ml min^{-1} per 1.73 m^2)	71.6±21.2	70.7±20.6	79.7±21.7	<0.001
$eGFR_{cys}$ (ml min^{-1} per 1.73 m^2)	82.3±24.1	81.4±24.0	85.1±24.3	0.003
$eGFR_{average}$ (ml min^{-1} per 1.73 m^2)	77.0±21.6	76.1±21.4	82.4±21.8	<0.001
CKD (%, using $eGFR_{cr}$)	43	51	47	0.005
CKD (%, using $eGFR_{cys}$)	39	45	45	0.04
CKD (%, using $eGFR_{average}$)	40	47	46	0.04

Abbreviations: BMI, body mass index; Cr, creatinine; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; $eGFR_{cr}$, creatinine-based eGFR; $eGFR_{cys}$, cystatin C-based eGFR; $eGFR_{average}$, average eGFR; HbA_{1c}, hemoglobin A_{1c}; RAS, renin–angiotensin system; UACR, urinary albumin–creatinine ratio.
Data are expressed as mean±s.d. or percentage.
Data for UACR are expressed as the median (interquartile range).

and with hypertension tended to be lower in the never smokers and current smokers compared with the past smokers. The serum creatinine levels decreased in the current smokers, whereas there was no significant difference in the serum cystatin C levels between the groups. The mean UACR tended to be increased in the past and current smokers, and the mean eGFR was increased in the current smokers, irrespective of the equations. The proportion of participants with CKD was likely to be high in the past and current smokers.

The clinical characteristics of the study participants according to the number of cigarettes smoked per day are shown in Supplementary Table S1. The mean age, the duration of diabetes and the proportion of participants with regular exercise habits decreased, whereas the BMI, the HbA_{1c} and the proportion of participants with insulin treatment increased with an increase in cigarettes per day. The serum creatinine decreased, whereas the UACR, $eGFR_{cr}$, $eGFR_{cys}$ and $eGFR_{average}$ increased with the number of cigarettes per day. The proportion of participants with CKD did not exhibit a distinctive tendency.

When the current smokers were subdivided by the Brinkman index (Supplementary Table S2), the mean age and duration of diabetes tended to increase with an increase in the Brinkman index; however, the never smoker cohort exhibited the highest values. The current drinking habit and HbA_{1c} increased, whereas the regular exercise habit decreased in association with an increase in the Brinkman index. In conjunction with an increased Brinkman index, the serum creatinine decreased and the UACR increased. The mean eGFR of the current smokers was increased compared with the never smokers, whereas the participants with a Brinkman index of less than 600 exhibited the highest mean value. The frequency of CKD was highest among the participants with a Brinkman index of $\geqslant 1000$.

Regarding the years since quitting smoking (Supplementary Table S3), the mean age, duration of diabetes and total protein intake increased; in contrast, the duration of smoking, number of cigarettes per day, Brinkman index, BMI and HbA_{1c} decreased with the years since quitting. There were no apparent trends in the serum creatinine, cystatin C, UACR or the proportion of participants with CKD; however, the eGFR tended to decrease with the years after cessation, irrespective of the equations used.

Figure 1 indicates the age-adjusted proportions of patients with CKD defined as albuminuria and/or an eGFR <60 ml min^{-1} per 1.73 m^2 (ref. 5) according to the smoking status. In the analysis using the $eGFR_{cr}$, the proportion of patients with CKD tended to increase in parallel with an increasing number of cigarettes smoked per day in the current smokers compared with the never smokers (Figure 1a, left, P for trend $=0.007$). A similar tendency was also identified for the Brinkman index, which reflects a cumulative amount of cigarettes (Figure 1a, center, P for trend $=0.003$). Regarding the association with the years since quitting cigarette smoking, those with CKD tended to decrease with the years after smoking cessation (Figure 1a, right,

P for trend =0.004). CKD defined using the $eGFR_{cys}$ and $eGFR_{average}$ also increased with the amount of smoking (Figures 1b and c, left and center, *P* for trend <0.001 for all), whereas it decreased with increases in the years after smoking cessation (Figures 1b and c, right, *P* for trend <0.001 for both). These significant increases and reductions with the smoking status were substantially unchanged after adjusting

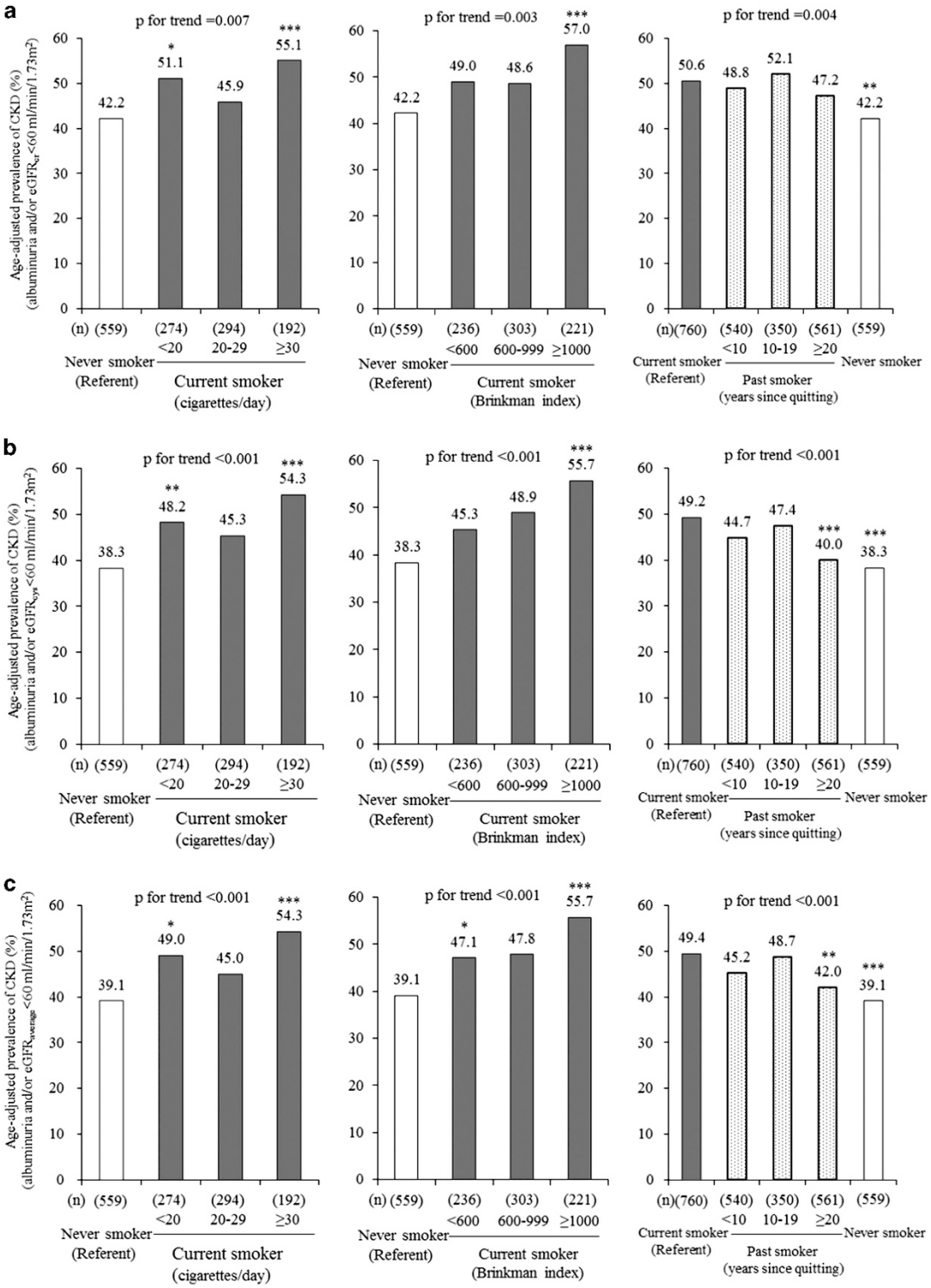

Figure 1 Age-adjusted proportions of patients with CKD defined as albuminuria (≥30 mg g^{-1} creatinine) and/or an eGFR <60 ml min^{-1} per 1.73 m^2 according to the smoking status in male patients. (**a**) eGFR was based on the serum creatinine ($eGFR_{cr}$), (**b**) eGFR was based on the serum cystatin C ($eGFR_{cys}$) and (**c**) eGFR was based on the average of the $eGFR_{cr}$ and $eGFR_{cys}$ ($eGFR_{average}$). CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; $eGFR_{cr}$, creatinine-based eGFR; $eGFR_{cys}$, cystatin C-based eGFR; $eGFR_{average}$, average eGFR; UACR, urinary albumin-creatinine ratio. **P*<0.05, ***P*<0.01, ****P*<0.001 *vs.* referent.

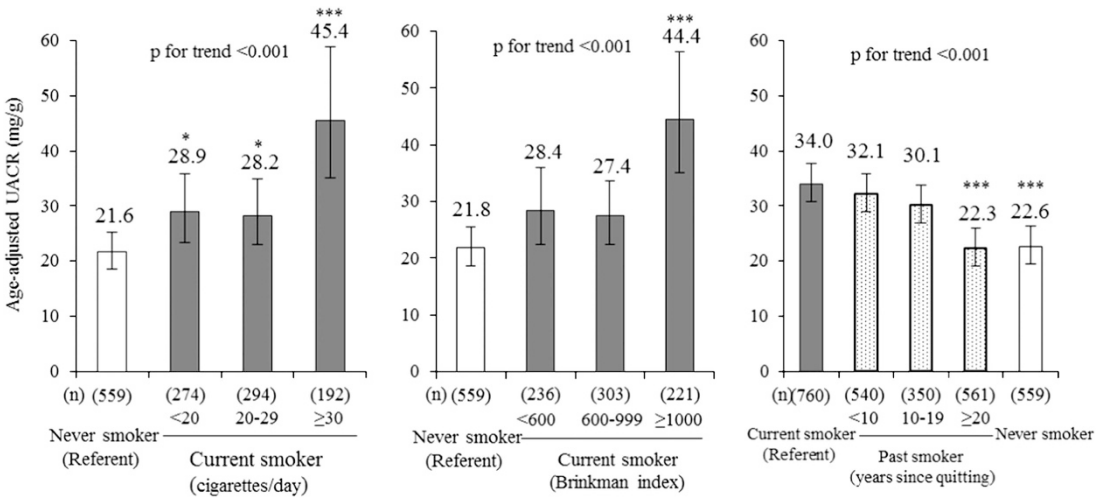

Figure 2 Age-adjusted mean UACR according to the smoking status in male patients. UACR, urinary albumin–creatinine ratio. UACRs are presented as geometric means. The bars represent 95% CIs. $*P<0.05$, $***P<0.001$ *vs.* referent.

for the confounding factors, including age, duration of diabetes, total energy intake, total protein intake, current drinking habits, regular exercise habits, depressive symptoms, BMI, HbA_{1c}, SBP and use of renin–angiotensin system inhibitors.

Figure 2 shows the age-adjusted mean values of the UACR according to the smoking status. The UACR levels significantly increased with increases in the amount of smoking in the current smokers compared with the never smokers (Figure 2, left and center, P for trend <0.001 for both). However, it decreased linearly with increasing years after quitting (Figure 2, right, P for trend <0.001). Adjustment for the previously described confounders did not change these trends. Furthermore, these relations persisted even after an additional adjustment for the $eGFR_{average}$.

The associations of the smoking status with the eGFR were subsequently evaluated (Figure 3). The age-adjusted $eGFR_{cr}$ significantly increased in parallel with increases in both the number of cigarettes per day and the Brinkman index, compared with the never smokers, in a dose–response manner (Figure 3a, left and center, P for trend <0.001, for both), whereas it decreased after smoking cessation (Figure 3a, right, P for trend $=0.001$). In contrast, the age-adjusted $eGFR_{cys}$ indicated an inverse association with the $eGFR_{cr}$; the $eGFR_{cys}$ decreased in association with the amount of smoking (Figure 3b, left and center, P for trend $=0.04$ and 0.02, respectively), whereas it increased as the years since quitting increased (Figure 3b, right, P for trend <0.001). However, the $eGFR_{average}$ did not significantly change according to the amount of cigarette consumption or the years since smoking cessation (Figure 3c). The multivariate-adjustment did not notably alter these tendencies.

Finally, we conducted the same analyses as shown in Figures 1–3 in 2095 female participants (Supplementary Figure S1). As a result of the small number of female current ($n=142$) and past ($n=173$) smokers, they were subdivided into two groups. The trends identified in the results obtained in the female participants were nearly the same as the trends identified in the male participants; however, they were not statistically significant.

DISCUSSION

Our study clearly demonstrated a dose- and time-dependent association between smoking habits and CKD in male Japanese patients with type 2 diabetes. However, the relationship between the smoking status and the eGFR varied according to the equations used to estimate the GFR. The $eGFR_{cr}$ increased, the $eGFR_{cys}$ decreased and the $eGFR_{average}$ did not change as the amount of smoking increased. In addition, inverse associations with years since quitting smoking were identified; both the UACR and $eGFR_{cr}$ decreased and the $eGFR_{cys}$ increased with years after smoking cessation. These associations were substantially unchanged following a multivariate adjustment for confounding factors, including age, BMI, lifestyle factors, and glycemic and blood pressure control. The inverse association with smoking cessation implies the reversibility of the harmful effects of smoking.

A number of epidemiological studies have examined the association between smoking and CKD.[6–8] However, in addition to a difference in the definition of CKD across the studies, the analyses conducted on each component of CKD, including proteinuria or a decreased glomerular filtration rate, have yielded inconsistent results. Regarding proteinuria, a positive association with current smoking habits has consistently been reported in most cross-sectional[6,10,14,15] and longitudinal[8,11–13,16] studies reviewed. A dose–response relationship with the amount of smoking has also been demonstrated.[10–13,15] The results of the present study indicated a graded increase in the UACR with both the number of cigarettes per day and the Brinkman index and support the hypothesis of a dose–response association. Smoking induces a state of increased oxidative stress as a result of the toxic chemicals contained in cigarettes, which leads to endothelial dysfunction and albuminuria.

Regarding the association with renal function, the majority of longitudinal studies have indicated an increased risk of decreased renal function in current smokers,[7,8,13,16,17] with the exception of two studies.[12,13] In contrast, the findings of the cross-sectional studies regarding renal function have been diverse, indicating an increased or decreased relationship with current smoking. Some studies have demonstrated that current smoking habits were associated with increased levels of GFR or creatinine clearance in the general population,[6,10,12,14] whereas other studies have demonstrated decreased GFR levels in current smokers among the general population[7,15] and diabetic patients.[17] Based on these results, the existence of smoking-associated glomerular hyperfiltration, especially at an earlier stage, which predisposes and consequently results in renal dysfunction at a later stage, is presumed. However, it should be noted that the previously discussed studies calculated the eGFR on the basis

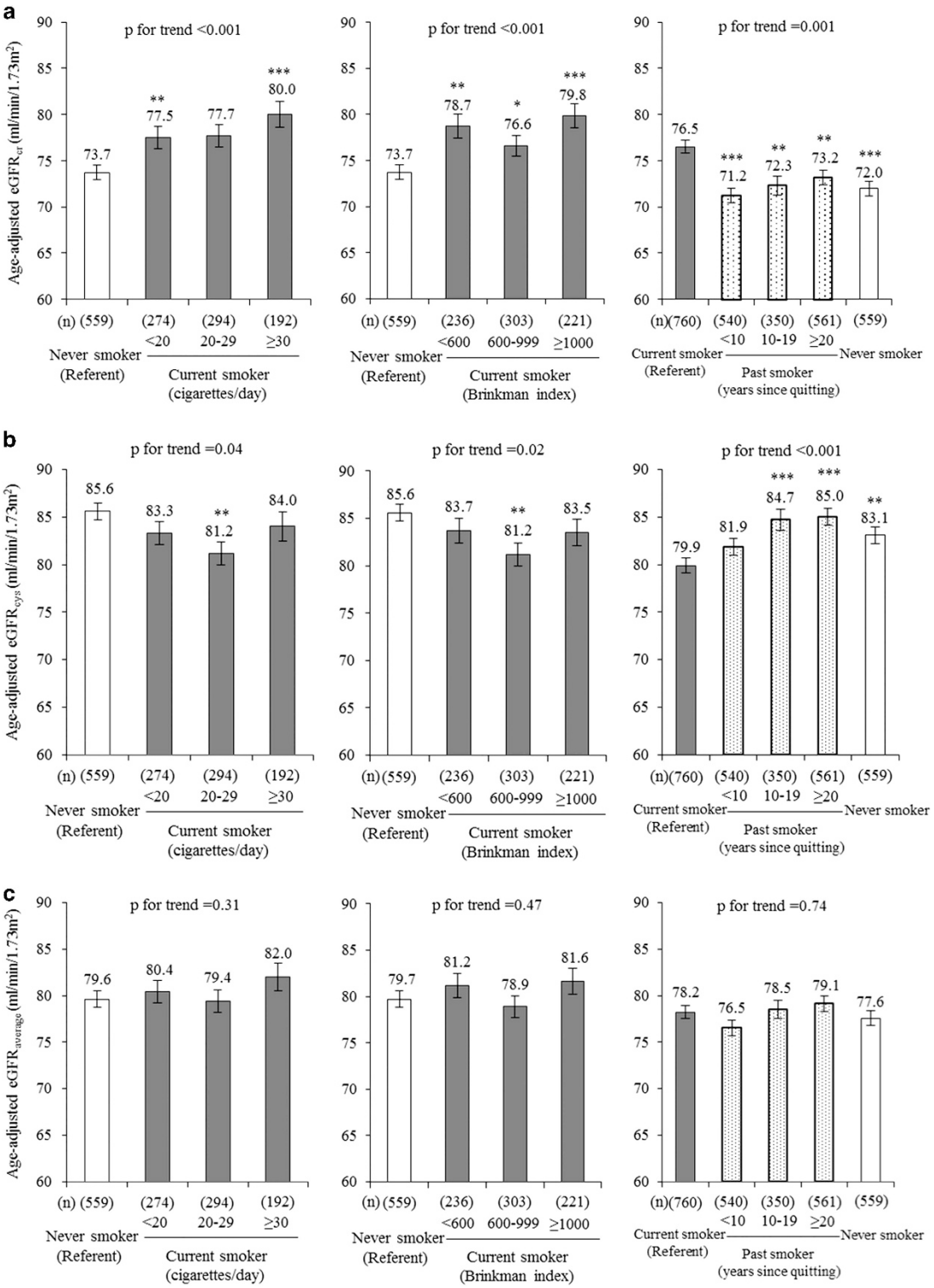

Figure 3 Age-adjusted mean eGFR according to the smoking status in male patients. (**a**) $eGFR_{cr}$, (**b**) $eGFR_{cys}$ and (**c**) $eGFR_{average}$. eGFR, estimated glomerular filtration rate; $eGFR_{cr}$, creatinine-based eGFR; $eGFR_{cys}$, cystatin C-based eGFR; $eGFR_{average}$, average eGFR. The bars represent the s.e. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs.* referent.

of the serum creatinine level; thus, caution is necessary when interpreting these findings because of the non-renal factors that affect the creatinine levels. Decreased muscle protein synthesis in smokers[34] and low serum albumin levels[35] in smokers followed by an increased tubular secretion of creatinine[36] may subsequently lead to lower serum creatinine levels and an increased $eGFR_{cr}$. Therefore, it is possible that the $eGFR_{cr}$ overestimates renal function in smokers.

Moreover, when evaluating the association with the eGFR using cystatin C, the direction of the results was opposite. However, current smoking habits were associated with increased serum cystatin C levels

independent of renal function as a result of unknown mechanisms,[37] which suggests the possibility of underestimation in smokers. A study conducted in the general Japanese population[38] demonstrated that the $eGFR_{cys}$ was lower in smokers compared with never smokers, whereas the $eGFR_{cr}$ was increased in smokers. It also indicated that there was no significant difference in the $eGFR_{average}$. Our study indicated a similar tendency to these findings; active smoking exhibited a positive association with the $eGFR_{cr}$, a negative association with the $eGFR_{cys}$ and a non-significant association with the $eGFR_{average}$. Therefore, we should consider the possibility of these overestimations and underestimations in the evaluation of renal function in smokers. In contrast, the average of the $eGFR_{cr}$ and $eGFR_{cys}$ exhibited better precision and accuracy for estimating the GFR (an inulin renal clearance) than when calculated separately[26] and may exclude the influence of non-renal factors,[26] as previously discussed. Taken together, the $eGFR_{average}$ may be the most appropriate marker for renal function evaluation in smokers.

The strengths of the current study include the large number and ethnicity of the participants. The epidemic of diabetes[19,20] and the high smoking rate[18] in the Asian population may broaden the applicability of the findings. Another strength is the assessment of both cystatin C and creatinine, which enabled us to calculate the $eGFR_{cys}$ and $eGFR_{average}$ in addition to the $eGFR_{cr}$ and thus to evaluate the effect of non-renal factors. Furthermore, the uniform collection of blood and urine samples and the use of a standardized method for measurement reduced the possibility of measurement errors. Moreover, the graded relationship of CKD with the amount of smoking and the inverse graded relationship with years since quitting suggest the reversibility of the harmful effects of smoking on CKD. The precise mechanisms have not been clarified; however, smoking cessation decreases oxidative stress[39] and improves vascular endothelial function,[39,40] which suggests the reversibility of the effect of smoking from a pathophysiologic point of view.

Several limitations of our study should be considered. First, we did not obtain information regarding second-hand smoke exposure. Previous studies have indicated an association between second-hand smoke and an increased risk of diabetes,[41] increased blood pressure level,[42] albuminuria[43] and proteinuria;[44] thus, there is a possibility of confounding by second-hand smoke. However, second-hand smoke exposure in those who have never smoked would likely bias our results toward a null association. Therefore, the results of our study may underestimate the effects of the true association. Second, the trends in the results were similar between the male and female participants; however, statistically significant associations were not identified in most analyses in the female participants. The reason for this discrepancy is not clear; however, it may be because of the small number of female current and past smokers, 7 and 8%, respectively, which is attributed to the Japanese cultural background. Thus, the generalizability of our results to female diabetic patients may be limited. Third, the population of the present study was limited to patients with diabetes, which may also limit the generalization of our findings to other populations. However, a previous study conducted in the general population also indicated a gradual increase in the risk of CKD in the order of never, former and current smokers,[7] which supports the hypothesis of the reversibility of smoking effects and extends the applicability of the present findings to subjects without diabetes. Fourth, cause-and-effect relationships cannot be inferred as a result of the cross-sectional design of our study. Finally, there may be other residual confounding factors in addition to the factors included in the present study.

In conclusion, the present study demonstrated dose- and time-dependent associations between smoking and both CKD and the UACR; however, the association with the eGFR depended on the definition used. Nevertheless, the presence of a dose-dependent association of active smoking and a graded inverse association of the years since quitting with CKD enhance the merit of smoking cessation in patients with type 2 diabetes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contributions: TO, UN and MI were responsible for the study concept and design, conducted the analyses and drafted the manuscript, and HF, SK, HI, TJ, YK, YI, AS and TK contributed to data interpretation and discussion. All authors participated in critically revising the manuscript and approved the final version.

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Supplementary Information accompanies the paper on Hypertension Research website (http://www.nature.com/hr)