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The effects of continuing and discontinuing smoking on the development of chronic kidney disease (CKD) in middle-aged healthy workers

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Response to Reviewers:	see attachment

The effects of continuing and discontinuing smoking on the development of chronic kidney disease (CKD) in middle-aged healthy workers

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Key words: smoking, chronic kidney disease (CKD), proteinuria, estimated glomerular filtration rate (eGFR), healthy person

Abstract

Objectives: The strength of the association between smoking and the development of chronic kidney disease (CKD) in the healthy working population has not been established. Methods: A retrospective 6-year observational study in 4,121 male and 2,877 female workers who were free of primary kidney disease, diabetes mellitus, severe hypertension and CKD signs. Proteinuria was detected by a dipstick method and glomerular filtration rate (GFR) was estimated by the equation of the Japan Society of Nephrology. Results: Sixty males (1.5%) and 21 females (0.7%) developed proteinuria during the 6 years. Irrespective of sex, those who continued smoking showed an odds ratio (OR) of 2.52 with a 95% confidence interval (CI) of 1.50~4.25 for developing proteinuria while those who quit smoking showed an OR of 1.29 (CI: 0.48~3.42) in comparison with non-smokers adjusting for confounders. Four hundred and forty-three males (10.7%) and 356 females (12.4%) developed a GFR of less than 60 mL/min/1.73m^2 corresponding to stage III CKD. The continuing smokers showed a low OR of 0.74 (CI: 0.60~0.90) for developing the low GFR. Smokers showed a higher mean GFR than non-smokers. The reduction of GFR during the 6-year period was not different between smokers and non-smokers, but it was larger in those who developed proteinuria than in those who did not, irrespective of smoking. Conclusions: Continuing

smokers showed a 2-fold or higher risk of developing proteinuria. Discontinuing smoking substantially reduced the risk. Longer observations may be required to detect the smoking-induced risk of developing stage III CKD in the working population.

Introduction

Cigarette smoking has been demonstrated to cause chronic kidney disease (CKD) characterized by proteinuria and/or a reduced glomerular filtration rate (GFR), first in patients having a preceding renal dysfunction due to diabetes mellitus (DM), primary kidney diseases or hypertension, and then in persons from the general population in communities or workplaces who did not have such renal dysfunction [1-4]. The strength of the association between smoking and the development of CKD in the generally healthy population, however, has not yet been fully established because of the vast heterogeneity in the source populations and the outcome measurements used in the previous studies [5].

On the other hand, the number of patients with end-stage kidney disease (ESKD) requiring kidney transplantation or hemodialysis has been increasing rapidly all over the world, from 430,000 to 2,100,000, nearly 5-fold in the last two decades. In Japan, some 300,000 patients are undergoing dialysis therapy, while some 13,300,000 CKD patients are estimated to be pre-ESKD [6]. Therefore, the prevention of CKD should be targeted in health promoting activities as a common disease in the general population including those in workplaces. However, the strength of the association between smoking and the development of CKD is vague especially in working populations [7] that are mainly composed of middle-aged adults and probably less vulnerable to the toxic effects of

smoking than community populations composed of many elderly persons aged 60 years or older.

Furthermore, many of the previous studies in workplaces were cross-sectional in design [8-12]. A few longitudinal studies conducted in workplaces were so far limited to a small number of subjects [13] or a short period of observation [14]. Therefore, further studies are required to determine the strength of the association in long-term and large-scale observations on populations recruited from workplaces. In the present study, the data of 4,121 male and 2,877 female generally healthy workers obtained from the annual health check-ups conducted in various kinds of workplaces at a 6-year interval were analyzed to clarify the effects of continuing and discontinuing smoking on the development of CKD signs of proteinuria and/or a reduced GFR.

Subjects and methods

The subjects were 7,964 workers (4,637 males and 3,327 females) recruited from a total of 20,782 in 447 various kinds of workplaces located in Ishikawa prefecture, Japan, not in a randomized fashion. The 20,782 workers had their serum creatinine (Cr) concentrations measured in 2003 as part of the annual health check-ups in workplaces conducted by an occupational health service organization, and 7,964 (38.3%) of them

had it measured again in 2009 by the same organization. Many young workers of the original population were not tested in 2009. However, nearly 70% of the workers aged 40 years or older undertook the test in 2009.

Examinations in the health check-ups

The examinees were asked to refrain from smoking for 30 min or longer before the beginning of the examinations. Height and weight were measured with jacket and shoes removed, and 0.5 kg subtracted in April to September and 1.0 kg in October to March for the net body weight. Body mass index (BMI) was calculated as the net body weight (kg) / height (m)^2. Blood pressure (BP) was measured by experienced nurses with an automatic manometer, UM-15P, Parama-Tech Co., Fukuoka, Japan, in the sitting position after resting on a chair for 5 min or longer. The Japanese Industrial Standard-sized arm-cuff was used except for persons having a thick arm girth of 34 cm or above for whom a larger one used. The cuff position was maintained at the heart level during the measurement. BP (mmHg) was graded into 6 levels of "optimal BP (＜120 and ＜80)", "normal BP (＜130 and ＜85)", "high-normal BP (130-139 and/or 85-89)", "grade I hypertension (140-159 and/or 90-99)", "grade II hypertension (160-179 and/or 100-109)" and "grade III hypertension (≧180 and/or ≧110)" according to the guidelines of the Japanese Society of Hypertension (JSH) [15], and scored 1 to 6 for the

BP levels. Urine samples were analyzed immediately after collection for protein concentration semi-quantitatively using a dipstick of Uro-paper III distributed by Eikenkagaku Co., Tokyo, Japan.

Venous blood samples were ice-cooled immediately after collection and conveyed to a laboratory for analyses within 4 hours. Hemoglobin A1c (HbA1c) level (%) in blood was measured by the previous standard methods proposed by Japan Diabetes Society (JDS) using an automatic analyzer of HLC-723G8, Tosoh Co., Tokyo, Japan. It was then estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value by the formula: HbA1c (NGSP) = HbA1c (JDS) + 0.4 [16]. Plasma samples were analyzed for glucose concentration (PG) using an automatic analyzer of GA1170, Arkray Inc. Kyoto, Japan. Fasting PG of 126 mg/dl, postprandial PG of 200 mg/dl, and/or HbA1c of 6.5% or higher were diagnosed as DM. Further, fasting PG of 110 mg/dl, postprandial PG of 140 mg/dl, and/or HbA1c of 5.8% or higher were defined as impaired glucose regulation (IGR).

Serum samples were analyzed for lipid concentrations such as total cholesterol (Tchol), low density lipoprotein cholesterol (LDLc), high density lipoprotein cholesterol (HDLc) and triglycerides (TG) using an automatic analyzer, HITACHI-7700, Hitachi High-Technology Co., Tokyo, Japan. Subjects were defined to have dyslipidemia such

as high cholesterolemia (hChol) when they showed Tchol of 220 mg/dl or above or LDLc of 140 mg/dl or above, low HDL cholesterolemia (lHDLc) when they showed HDLc of less than 40 mg/dl, and high triglyceridemia (hTG) when they showed TG of 150mg/dl or above in fasting serum or 250 mg/dl or above in postprandial serum. Serum Cr concentration was also measured by the automatic analyzer with an enzyme assay kit of N-Assay L CRE-K, Nittobo Medical Co., Tokyo, Japan. Then, GFR was estimated (eGFR) using the equation proposed by the Japan Society of Nephrology (JSN) as follows: $eGFR=194 \times (Age\ (year)^{-0.287}) \times (Cr\ (mg/dl)^{-1.094})$ [x 0.739 if female] [17].

The definition of CKD stages

The organization of Kidney Disease: Improving Global Outcomes (KDIGO) [18] classified the levels of GFR as follows: GFR of 90 mL/min/1.73m^2 or above was "normal", 60~89.9 was "mildly depressed", 30~59.9 was "moderately depressed", 15~29.9 was "severely depressed", and less than 15 indicated "renal failure level". Further, they defined the stages of CKD as follows: stage I; kidney damage (pathological abnormalities and/or abnormalities in blood, urine and imaging tests) with normal GFR, stage II; kidney damage with mildly depressed GFR, stage III; moderately depressed GFR irrespective of kidney damage, stage IV; severely depressed GFR, and stage V; renal failure level GFR. No kidney damage with a GFR of 60 mL/min/1.73m^2

or above was defined as “free of CKD”.

The subjects in the present study were categorized into CKD stages following the KDIGO’s definitions for further analyses. However, it should be noted that the categorization in the present subjects was determined by single measurements of proteinuria and eGFR although the clinical definition of CKD requires the detection of the CKD signs continuing for 3 months or longer. Among the subjects with “normal” eGFR, those showing an eGFR of 105 mL/min/1.73m^2 or higher, the upper 5% of the distribution in the present subjects, were defined as having an “elevated” eGFR. The subjects who showed proteinuria with an elevated eGFR as well as those with a normal eGFR were categorized into stage I CKD. None of the present subjects showed a low eGFR corresponding to stage V CKD or the renal failure level.

The definition of smoking status

The data of cigarette and alcohol consumption were obtained by interview by experienced nurses as mentioned in our previous articles [9, 11]. Smoking habits were classified into 4 categories, i.e., lifelong non-smokers, ex-smokers, current smokers consuming up to 1 pack a day, and smokers consuming more. For the sake of further analyses, the subjects were then categorized into 3 groups of smoking status: those who smoked at the 2009 health check-ups were defined as “continuous smokers” irrespective

of smoking status in 2003 although most of them had smoked in 2003 as well, those who did not smoke in either 2009 or 2003 irrespective of past smoking before 2003 were defined as “non-smokers”, and those who had smoked in 2003 but did not smoke in 2009, thus quitting at any time from 2003 to 2009, were defined as “ex-smokers”.

Usual alcohol consumption was categorized into 4 levels, i.e., essentially “non-drinkers” who did not drink alcoholic beverages during the last year or drank but less than once a month on average, “mild drinkers” who drank once a month or more but less than 70 mL of ethanol per week on average, “moderate drinkers” who consumed 70~209 mL per week on average and “heavy drinkers” who consumed 210 mL or more per week. They were scored 1 to 4 for alcohol consumption.

The subjects were further classified into 5 categories of occupation, i.e., clerks (category 1), managers or professionals (category 2), machine operators or motor-vehicle drivers (category 3), service or sales workers (category 4), and other occupations including security guards, farmers and unclassified jobs (category 5).

Data analyses

Of the 7,964 subjects, those whose data of body weight (37 males and 50 females), urinalyses (2 males and 114 females), or PG (7 males) were not available were excluded. Exclusions were also made for 13 males and 5 females who declared a past

history of primary kidney disease such as glomerulonephritis or nephrotic syndrome, and 252 males and 50 females who showed a high PG consistent with the diagnostic criteria of DM, and 28 males and 12 females who showed severe (grade III) hypertension (BP level 6) at the 2003 health check-ups. Among the remaining 4,298 males and 3,096 females, those who showed CKD signs at the 2003 health check-ups were excluded, i.e., 51 males and 19 females showing proteinuria and 121 males and 112 females showing an eGFR of less than 60 mL/min/1.73m^2. In addition, 5 males and 88 females who did not undergo urinalyses at the 2009 health check-ups were excluded. Finally, the data of 6,998 workers (4,121 males and 2,877 females, 87.9% of the original subjects) who were free of health problems markedly affecting renal function and CKD signs in 2003 were defined as generally healthy subjects and analyzed for the association between smoking status from 2003 to 2009 and the development of CKD in 2009.

All statistical analyses were performed using a package of IBM SPSS Statistics Ver.19 distributed by IBM Japan, Tokyo. Simple associations between the 3 categories of smoking status and the development of various stages of CKD in male and female workers were tested by χ^2-tests. Multiple logistic regression (MLR) analyses were performed to examine the independence of the associations after adjusting for the

effects of possible confounders such as sex, age, BMI levels of 1-4, BP levels of 1-5, alcohol consumption levels of 1-4, the presence of IGR and dyslipidemia, and the 5 categories of occupation. The changes in eGFR from 2003 to 2009 were compared in the male and female workers belonging to the 3 groups of smoking status and between those developing or not developing proteinuria in 2009 using generalized linear model (GLM) analyses adjusting for the confounders.

This study that analyzed the data anonymized in an unlinkable fashion provided by an occupational health service organization was approved by the ethics committee of Kanazawa Medical University.

Results

The profiles of 4,121 male and 2,877 female workers at the 2003 health check-ups are summarized in Table 1. The means, standard deviations (SDs) and the ranges of age (years) in the male and female workers were 41.2, 9.7 and 18~71, and 42.3, 9.3 and 18~69, respectively, and mostly 30~59 years, middle-aged in both sexes. The means, SDs and the ranges of BMI in the male and female workers were 23.2, 3.1 and 15.5~43.8 and 21.7, 3.1 and 13.2~41.1, respectively. Prevalence of overweight and obesity, grades I and II hypertension, and abnormal blood biochemistry tests of IGR,

hChol, lHDLc and hTG are shown in the table. The means, SDs and the ranges of eGFR (mL/min/1.73m^2) in the male and female workers were 83.3, 12.9 and 60~228, and 84.2, 14.5 and 60~198, respectively, and slightly higher in females.

Smoking status and development of CKD

Proteinuria was detected in 60 males (1.5%) and 21 females (0.7%) in the 2009 annual health check-ups. A "moderately depressed" eGFR of 30~59.9 mL/min/1.73m^2 corresponding to stage III CKD was detected in 443 males (10.7%) and 356 females (12.4%). More marked reduction of GFR corresponding to stage IV or V CKD was not found in any of the subjects. As shown in Table 2, male continuous smokers developed more frequently stages I and II CKD (1.6%) than non-smokers (0.8%) and ex-smokers (1.1%). On the other hand, the continuous smokers showed a lower development of stage III CKD (8.2%) than the non-smokers (12.8%) and ex-smokers (12.8%). The distributions of the CKD stages in the 3 groups of smoking status were significantly different (χ^2 test: $p<0.01$), and stage III CKD was significantly lower ($p<0.01$) in the male continuous smokers. In female workers, stages I and II CKD were also found more frequently in the continuous smokers (1.4%) than in non-smokers (0.4%) and ex-smokers (0.0%). Stage III CKD was similarly found in the non-smokers (12.5%), ex-smokers (10.2%) and continuous smokers (11.8%). The distributions of the CKD

stages were significantly different (p=0.04) in the 3 groups of smoking status but not different (p=0.81) for the development of stage III CKD in females.

For the sake of convenience for further discussion, the associations of the smoking status with the development of proteinuria, instead of stage I and II CKD, and those with the development of a low eGFR of 30~59 mL/min/1.73m^2 (stage III CKD), were examined for independence by MLR analyses adjusting for the possible confounding factors. In addition, "elevated" eGFR (hGFR) was included in the variables to be examined for the effect on the future development of proteinuria and low GFR. The results are shown in Table 3. Sex and the presence of hChol and lHDLc as well as the 5 categories of occupation were not significantly related to either the development of proteinuria or low eGFR. As for proteinuria, continuous smokers showed a significantly high odds ratio (OR) of 2.52 with a 95% confidence interval (CI) of 1.50~4.25, and ex-smokers showed an OR of 1.29 (CI: 0.48~3.42) in comparison with non-smokers. In addition, BMI and BP levels as well as the presence of IGR showed significant promoting effects on the development of proteinuria. hGFR showed no significant effects on the developments of proteinuria.

As for low eGFR, continuous smokers showed a significantly low OR of 0.74 (CI: 0.60~0.90) and ex-smokers showed an OR of 1.05 (CI: 0.78~1.41) in comparison

with non-smokers. Age, BMI and hTG showed significant promoting effects on the development of low eGFR while alcohol consumption showed a significant protective effect. In addition, hGFR showed a strong protective effect on the development of low eGFR with an OR of 0.05 (CI: 0.01~0.22).

Table 4 shows the results of MLR analyses in the subjects excluding those with IGR, grade I and II hypertension and obesity (BMI≧30). In the subjects of A excluding those showing IGR in 2003, the OR of proteinuria in the continuous smokers was decreased to 2.45 (CI: 1.39~4.32) but remained significant. In the subjects of B further excluding those showing grade I and II hypertension, the OR was decreased to 2.14 (CI: 1.03~4.46) but significant. In the subjects of C further excluding obese subjects with a BMI of 30 or above, the OR of 1.94 (CI: 0.89~4.26) was not significant. In these analyses hChol was shown to be a significant factor promoting the development of proteinuria rather than BP and BMI levels. No marked changes were observed in the association between continuous smoking and the low incidence of low eGFR even after excluding the subjects with IGR, hypertension and obesity.

Smoking status and the reduction of GFR

Table 5 shows the means of eGFR at the 2003 and 2009 health check-ups and the changes during the 6-year period in the male and female workers of the 3 groups of

smoking status. Female workers showed a significantly higher mean eGFR than male workers in both 2003 and 2009. However, the 6-year changes, 10.6 and 11.0 mL/min/1.73m^2 in male and female workers respectively, were not significantly different. Male ex-smokers and continuous smokers, most of whom had smoked in 2003, showed a significantly higher mean eGFR than non-smokers in 2003. In 2009, continuous smokers still showed a higher eGFR than non-smokers, but ex-smokers no longer showed a higher eGFR. A significantly higher eGFR was observed in female ex-smokers but not in the continuous smokers in 2003. The reduction of eGFR during the 6-year period was significantly larger in ex-smokers than in non-smokers and continuous smokers in males. In females, the reduction of eGFR was largest in ex-smokers similarly to males but the differences were not significant.

Although the results are not shown in the table, a generalized linear model (GLM) analysis was performed in the male and female workers to examine the independent effects of development of proteinuria in 2009 as well as smoking status on the reduction of eGFR in the 6-year adjusting for the possible confounders. The interactive effect between the development of proteinuria and the smoking status on the eGFR values was not significant in either sex and thus not included in the model equations. Male workers who developed proteinuria showed a significantly larger reduction of eGFR in

comparison with those who did not develop proteinuria (p=0.005) by 3.41 mL/min/1.73m^2 irrespective of smoking status. In female subjects, those who developed proteinuria also showed a lager reduction of eGFR in comparison with who did not, by 2.66 mL/min/1.73m^2. The difference was, however, not significant.

Discussion

A high risk of proteinuria in smokers

This 6-year observation in mostly middle-aged, generally healthy male and female workers who had been free of CKD signs demonstrated that those who continued smoking during the period, irrespective of sex, had a 2.5-fold higher risk of developing proteinuria than those who did not smoke. Those who had quit smoking showed a lower, nearly half, risk. These findings confirmed that chronic smoking is a cause of developing CKD, mainly stage I or II CKD, even in generally healthy middle-aged persons in workplaces and that quitting smoking is beneficial for substantially reducing the risk.

The further analyses in the subjects excluding those with IGR, grade I and II hypertension and obesity showed that the development of proteinuria was strongly related to those major cardiovascular risk factors. These results imply that the

prevention of CKD in the general population should be performed by the combined efforts of improving other lifestyle factors and the proper use of medicines improving metabolism and BP control as well as the cessation of smoking. At the same time, the results of those analyses suggested that the risk of developing proteinuria was at least 2-fold in entirely healthy middle-aged smokers. A recently published study on 10,118 Japanese male workers aged 40~55 years [19] showed a 1.5-fold risk of proteinuria in smokers during the 6-year observation. The somewhat higher risk in the present study may have reflected the presence of subjects aged 56 years or older.

The risk of low GFR in smokers

Smokers showed a significantly lower risk of developing stage III CKD with a GFR of less than 60 mL/min/1.73m^2, in comparison with non-smokers. Smokers tended to have a higher GFR than non-smokers. The higher GFR and lower risk of low GFR in smokers were consistent with the previous findings in general populations especially from workplaces [8-10, 12]. The present study confirmed that the elevated GFR in smokers was caused by smoking since a large reduction of GFR was observed after its cessation. The ex-smokers, however, did not show an excessive risk of developing a low GFR, suggesting that the reduction of GFR after quitting smoking does not mean impaired renal function.

The present male and female workers showed eGFR reductions of 10.6 and 11.0 mL/min/1.73m^2 respectively on average during the 6 years. Although the reduction of eGFR was not different between smokers and non-smokers, the subjects who developed proteinuria during the period showed a larger reduction of eGFR than those who did not irrespective of smoking, by 3.41 and 2.66 mL/min/1.73m^2 in males and females respectively, although a significant difference was detected only in males. These findings suggested that although GFR in smokers was even higher than in non-smokers, the GFR would finally decline more in smokers since the risk of developing proteinuria was 2-fold or higher in smokers. This contention should be examined in further studies.

The fate of elevated GFR in smokers

Yoon et al. [12] proposed that most smokers in the healthy population, including those showing an elevated GFR, would not show any renal functional deterioration in their lifetime, while only a small, susceptible subset of the population would show both a low GFR and proteinuria from the findings in a cross-sectional study in mostly middle-aged participants of a large scale health screening program in Korea. Namely, the association of smoking with GFR was entirely different between those showing an GFR of 50 mL/min/1.73m^2 or above and those with a lower GFR, i.e., a higher GFR in smokers was observed only among the subjects showing the higher GFR, but smokers

inversely tended to have a lower GFR among the subjects with lower GFR. However, the inverse association of smoking with eGFR between those showing the higher and lower eGFR was not confirmed in the present subjects, i.e., eGFR was higher in smokers than in non-smokers in both the subjects showing an eGFR of 60 mL/min/1.73m^2 or above and those showing a lower eGFR. The contention of Yoon et al. thus requires confirmation by further studies.

On the other hand, the elevated GFR in smokers might be an early sign of renal dysfunction. Orth and Hallan [2] have extensively discussed possible mechanisms underlying the development of CKD in smokers such as toxicity of heavy metals in tobacco smoke [20-22], intrarenal vasoconstriction [23-27], and oxidative stress and inflammatory process [28-30]. Based on the relatively copious evidence in the previous studies, glomerular hypertension due to intrarenal vasoconstriction seems the most plausible mechanism, which is related to a stimulated renin-angiotensin-aldosterone system (RAAS) caused by nicotine inhaled with cigarette smoke. The elevation of GFR via a stimulated RAAS was confirmed experimentally in dogs to which nicotine was administered [31], and it may be a sign of glomerular hyperfiltration and early stage renal damage like that observed in diabetic nephropathy [32]. However, in the present study, an elevated GFR in smoker was not related to the future development of

proteinuria and low GFR. This contention, therefore, has not been confirmed either. The fate of elevated GFR in smokers should be elucidated by longer-term observations.

Mild alcohol consumption showed a significant protective effect on the reduction of GFR, which was consistent with the finding in our previous cross-sectional study [9]. The anti-atherogenic property of mild alcohol consumption [33-35] may have even acted protectively on renal function.

Limitations of the present study

In addition to the limitations related to the sensitivity of dipstick-measured proteinuria and the validity of eGFR using the JSN equation [9] and the single measurements of those parameters, the selection of the study subjects should be considered as limitations in the present study. Japanese companies are mandated to make their workers aged 35 years and 40 years or older undertake annual health check-ups including the blood tests of liver enzyme activities and serum lipid concentrations. The measurement of serum Cr is an option in the check-ups. The present subjects were thus assumed to work for such companies that can afford to have the workers undergo the measurements of serum Cr. On the other hand, most of the companies were small or medium-sized private enterprises. The present subjects were, therefore, not special but generally representative of the working population in Japan.

Of the total of 20,782 workers who had their serum Cr measured in 2003, only 7,964 (38.3%) had it measured again in 2009. Participation rates of the employees in the annual health check-ups were as high as 95% or more in most of the workplaces in the present study, and incidental drop-outs were not common. The majority of the drop-out must be young workers because of the optional choice of blood tests and elderly ones because of the mandatory retirement age. It is reasonable to assume that the elderly workers who remained in the study subjects were generally in better health than those who retired and dropped out from the study. Drop-outs due to sick leave or health-associated resignation from the workplaces may have occurred, and those in poor health must be more vulnerable to the toxic effects of smoking. To sum up, the unintentional drop-out subjects in this retrospective study are not considered to have exaggerated the strength of the association of smoking with the development of CKD.

It can be concluded that continuing smoking causes a 2-fold or higher risk of developing proteinuria in mostly middle-aged healthy workers in both sexes while quitting smoking substantially reduces the risk. This 6-year observation may be too short to reveal the risk of developing stage III or severer CKD in smokers from the working population.

Conflict of interest

This work was supported by KAKENHI, a Grant-in-Aid for Scientific Research (C), 2010, from the Japan Society for the Promotion of Science (JSPS).

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Reviewers' comments への回答

ご指摘ありがとうございました。以下のように改訂いたしました。

#1. The authors should indicate whether they used HbA1c of JDS value or NGSP value.

回答：”Subjects and methods”で、NGSP 値であることを P8L5 以下に次のように記載し、JDS の文献（16）を引用しました。本研究のデータ解析時点では NGSP=JDS+0.4 として処理しましたので、そのように記載しました。

Hemoglobin A1c (HbA1c) level (%) in blood was measured by the previous standard methods proposed by Japan Diabetes Society (JDS) using an automatic analyzer of HLC-723G8, Tosoh Co., Tokyo, Japan. It was then estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value by the formula: HbA1c (NGSP) = HbA1c (JDS) + 0.4 [16].

［Ref.16］ Kashiwagi A, Kasuga M, Araki E, Oka Y, Hanafusa T, Ito H et al. International clinical harmonization of glycated hemoglobin in Japan: from Japan Diabetes Society to National Glychohemoglobin Standardization Program values. Diabetol Int. 2012; 3: 8-10.

#2. Some part of Discussion is too long for this reviewer to follow the content. It should be shortened or reconstructed : 1) the reviewer could not follow the content of one paragraph in Discussion mentioned below, 2) limitation is too long, and 3) conclusion(s) should be mentioned in the last part of this paper to help readers to confirm the essence of the present paper.

回答①　論文全体について再度簡潔を心がけて書き直しました。特にご指摘の２）limitation を短縮し、３）最後に結論を書き加えました。

[Paragraph of Page20 line 3 from the bottom to Page 21 line 7 in Discussion] The male and female workers who had been free of CKD signs showed eGFR reductions of 10.6 and 11.0 mL/min/1.73m^2 respectively on average during the 6 years, but those who developed proteinuria during the period showed a larger reduction of eGFR of 3.41 and 2.66 mL/min/1.73 m^2 in males and females, respectively, than those who did not.

This reviewer could not find the correspond part of this paragraph (Discussion) in Result section. Moreover, the reviewer could not understand why reductions of eGFR among subjects with proteinuria (3.41 and 2.66 mL/min/1.73 m^2 in males and females) were larger than the figures of 10.6 and 11.0 mL/min/1.73m^2.

回答②　きわめて初歩的な英語の書き間違いにより、意味が取れなくなってしまいました。お詫び申し上げます。ご指摘の”but those who developed proteinuria during the period showed a larger reduction of eGFR of 3.41 and 2.66 mL/min/1.73 m^2 in males and

females, respectively, than those who did not."の文章中の" eGFR of 3.41 and 2.66 mL/min/1.73 m²"は、"eGFR by 3.41 and 2.66 mL/min/1.73 m²"の書き間違いです。タンパク出現者の GFR 低下がこの分だけ大きかったという意味で書くつもりが、未熟な前置詞の間違いを犯してしまいました。改訂稿では意味の取り違えがおこらないよう、以下のように書き換えました。(P20L1～L6)

The present male and female workers showed eGFR reductions of 10.6 and 11.0 mL/min/1.73m^2 respectively on average during the 6 years. Although the reduction of eGFR was not different between smokers and non-smokers, the subjects who developed proteinuria during the period showed a larger reduction of eGFR than those who did not irrespective of smoking, by 3.41 and 2.66 mL/min/1.73m^2 in males and females respectively, although a significant difference was detected only in males.

なお、この Discussion に対応する Results は P17L12 ～P18L4 の、" Although the results are not shown in the table, …."以下に、GLM 解析の結果として記載しています。

Associate editor:

回答：ご指摘ありがとうございました。ご指示にしたがって訂正いたしました。

#1. The abbreviation used for the first time should be spelled out: odds ratios (OR) and 95% confidence intervals (95% CIs).

回答："Abstract"の該当箇所（P3L9）を spell out しました。

#2. You should unify terms, because you used both "men/women" and "male/female" in this text.

回答："Subjects and methods"の冒頭（P6L14）の men/women を males/females に書き換えました。

Table

Table 1. Profiles of the subjects at the 2003 health check-ups

Variables		Men (n=4,121) No.	(%)	Women (n=2,877) No.	(%)
Age (years)	~29	587	(14.2)	344	(12.0)
	30~59	3,473	(84.3)	2,484	(86.3)
	60~	61	(1.5)	49	(1.7)
BMI	~18.4	161	(3.9)	356	(12.4)
	18.5~24.9	2,920	(70.9)	2,122	(73.8)
	25.0~29.9	928	(22.5)	348	(12.1)
	30.0~	112	(2.7)	51	(1.8)
BP (mmHg)	<120 and <80	1,245	(30.2)	1,629	(56.6)
	<130 and <85	1,419	(34.4)	739	(25.7)
	130-139 and/or 85-89	691	(16.8)	242	(8.4)
	140-159 and/or 90-99	631	(15.3)	227	(7.9)
	160-179 and/or 100-109	135	(3.3)	40	(1.4)
Blood chemistry test[a]	IGR	226	(5.5)	70	(2.4)
	hChol	978	(23.7)	650	(22.6)
	lHDLc	342	(8.3)	93	(3.2)
	hTG	755	(18.3)	48	(1.7)
eGFR[b]	105.0 ~	238	(5.8)	227	(7.9)
	90.0 ~ 104.9	900	(21.8)	535	(18.6)
	60.0 ~ 89.9	2983	(72.4)	2115	(73.5)
Cigarettes	Never smoke	1,028	(24.9)	2,539	(88.3)
	Ex-smoke	904	(21.9)	57	(2.0)
	Smoke up to 1 pack/d	1,540	(37.4)	273	(9.5)
	More	649	(15.7)	8	(0.3)
Alcohol	Usually not drink	1,246	(30.2)	2,088	(72.6)
	Up to 69 ml/w of ethanol	1,474	(35.8)	673	(23.4)
	70~209 ml/w	1,256	(30.5)	108	(3.8)
	More	145	(3.5)	8	(0.3)
Occupation	Clerks	1,000	(24.3%)	1,129	(39.2%)
	Managers / Professionals	1,144	(27.8%)	600	(20.9%)
	Operators / Drivers	1,223	(29.7%)	342	(11.9%)
	Service / Sales	636	(15.4%)	656	(22.8%)
	Others	118	(2.9%)	150	(5.2%)

a: For the definitions of blood chemistry tests, refer to text. b: mL/min/1.73m^2.

Table

Table 2. Development of CKD (Stage I~III)[a] during 6 years in generally healthy workers who were free of CKD signs beforehand

	Male workers				Female workers			
Smoking status[b]	No.	Stage I[c] No. (%)	Stage II[c] No. (%)	StageIII[c,d] No. (%)	No.	Stage I[c] No. (%)	Stage II[c] No. (%)	Stage III[c,d] No. (%)
Non-smokers	1,806	0 (0.0)	14 (0.8)	231 (12.8)	2,570	0 (0.0)	10 (0.4)	321 (12.5)
Ex-smokers	459	1 (0.2)	4 (0.9)	59 (12.8)	78	0 (0.0)	0 (0.0)	8 (10.2)
Continuous smokers	1,856	4 (0.2)	26 (1.4)	153 (8.2)	229	1 (0.4)	2 (1.0)	27 (11.8)
Total	4,121	5 (0.1)	44 (1.1)	443 (10.7)	2,877	1 (<0.1)	12 (0.4)	356 (12.4)

a: For the definitions of CKD stages, refer to text.

b: Non-smokers: those who had never smoked until 2009. Ex-smokers: those who had smoked in 2003 but quit smoking at any time between 2003 and 2009. Continuous smokers: those who continued smoking from 2003 to 2009 or started smoking at any time during the period.

c: The distributions of the CKD stages I~III among the 3 groups of smoking status were significantly different in both male and female workers (χ^2 =28.84, df=6, p<0.01 and $\chi 2$ =13.52, df=6, p=0.04, respectively).

d: Stage III CKD was significantly lower in male continuous smokers (χ^2 =22.11, df=2, p<0.01) but not different in females ($\chi 2$ =0.43, df=2, p=0.81).

Table

Table 3. Results of multiple logistic regression (MLR) analyses on the association of variables with the development of CKD signs

Variables[a]	Proteinuria Odds ratio (CI[c])	*p*	Low eGFR[b] Odds ratio (CI[c])	*p*
Sex (Women / Men)	1.06 (0.57 ~ 1.98)	0.859	1.02 (0.84 ~ 1.24)	0.866
Age (year)	1.02 (0.99 ~ 1.05)	0.134	1.08 (1.07 ~ 1.09)	<0.001
BMI levels (1~4)	1.82 (1.27 ~ 2.59)	0.001	1.37 (1.19 ~ 1.57)	<0.001
Blood pressure levels (1~5)	1.33 (1.10 ~ 1.62)	0.004	0.97 (0.90 ~ 1.04)	0.347
IGR	2.35 (1.19 ~ 4.65)	0.014	0.84 (0.59 ~ 1.18)	0.312
hChol	1.46 (0.90 ~ 2.36)	0.130	1.06 (0.90 ~ 1.27)	0.480
lHDLc	1.33 (0.64 ~ 2.79)	0.449	0.92 (0.65 ~ 1.29)	0.625
hTG	1.20 (0.68 ~ 2.13)	0.533	1.44 (1.14 ~ 1.80)	0.002
hGFR	1.35 (0.52 ~ 3.45)	0.538	0.05 (0.01 ~ 0.22)	<0.001
Smoking status (vs. Non-smokers)		0.002		0.006
Ex-smokers	1.29 (0.48 ~ 3.42)	0.614	1.05 (0.78 ~ 1.41)	0.735
Continuous smokers	2.52 (1.50 ~ 4.25)	0.001	0.74 (0.60 ~ 0.90)	0.003
Alcohol consumption levels (1~4)	0.87 (0.66 ~ 1.16)	0.349	0.87 (0.78 ~ 0.97)	0.009
Occupations (1~5)	1.01 (0.84 ~ 1.21)	0.941	0.98 (0.92 ~ 1.04)	0.433

a: For the definitions of the variables, refer to text. b: eGFR of less than 60 mL/min/1.73m^2. c: 95% confidence interval.

Table

Table 4. Results of multiple logistic regression (MLR) analyses on the association of variables with the development of proteinuria in the subjects excluding those with impaired glucose regulation ((IGR), grade I and II hypertension and obesity

Variables[d]	Subjects A[a] (Proteinuria 70) Odds ratio (CI[e])	*p*	Subjects B[b] (Proteinuria 43) Odds ratio (CI[e])	*p*	Subjects C[c] (Proteinuria 37) Odds ratio (CI[e])	*p*
Sex (Women / Men)	0.91 (0.47 ~ 1.75)	0.908	0.98 (0.44 ~ 2.18)	0.959	1.09 (0.47 ~ 2.56)	0.842
Age (year)	1.02 (0.99 ~ 1.05)	0.227	1.01 (0.98 ~ 1.05)	0.449	1.02 (0.98 ~ 1.06)	0.369
BMI levels	1.83 (1.26 ~ 2.66)	0.002	2.03 (1.24 ~ 3.33)	0.005	1.11 (0.56 ~ 3.72)	0.763
Blood pressure levels	1.37 (1.11 ~ 1.69)	0.003	0.87 (0.57 ~ 1.35)	0.547	0.87 (0.54 ~ 1.39)	0.555
hChol	1.54 (0.91 ~ 2.59)	0.108	2.15 (1.11 ~ 4.16)	0.023	2.37 (1.16 ~ 4.83)	0.017
lHDLc	1.45 (0.66 ~ 3.17)	0.352	1.40 (0.50 ~ 3.94)	0.525	1.20 (0.34 ~ 4.30)	0.776
hTG	1.20 (0.64 ~ 2.26)	0.563	1.23 (0.53 ~ 2.86)	0.627	1.47 (0.58 ~ 3.72)	0.418
hGFR	1.44 (0.56 ~ 3.72)	0.450	1.25 (0.37 ~ 4.23)	0.716	1.44 (0.42 ~ 4.93)	0.558
Smoking status		0.007		0.086		0.126
Ex-smokers	1.22 (0.41 ~ 3.60)	0.723	0.85 (0.19 ~ 3.80)	0.828	0.44 (0.06 ~ 3.42)	0.432
Continuous smokers	2.45 (1.39 ~ 4.32)	0.002	2.14 (1.03 ~ 4.46)	0.041	1.94 (0.89 ~ 4.26)	0.098
Alcohol consumption levels	0.89 (0.65 ~ 1.22)	0.459	0.79 (0.52 ~ 1.21)	0.275	0.75 (0.47 ~ 1.19)	0.217
Occupations	1.02 (0.83 ~ 1.24)	0.867	0.98 (0.76 ~ 1.26)	0.850	0.92 (0.69 ~ 1.21)	0.534

a: 3895 male and 2807 female workers without IGR. b: 3227 males and 2553 females without IGR and grade I+II hypertension.

c: 3165 males and 2521 females without IGR, grade I+II hypertension and obesity (BMI of 30 or above).

d: For the definitions of the variables, refer to text. e: 95% confidence interval.

Table

Table 5. Means (Ms) and standard deviations (SDs) of eGFR (mL/min/1.73m^2) and those of the change (Δ) during 6 years

Sex	Smoking status[a]	No.	2003 M ± SD	2009 M ± SD	Δ2003～2009 M ± SD
Men	Total	4,121	83.3 ± 12.9	72.6 ± 10.8	10.6 ± 9.2
	Non-smokers	1,806	81.7 ± 12.7	71.2 ± 10.4	10.4 ± 9.3
	Ex-smokers	459	83.9 ± 12.5 #	71.6 ± 10.1	12.3 ± 9.2 #
	Continuous smokers	1,856	84.6 ± 13.1 #	74.2 ± 11.1 #	10.4 ± 9.2 ##
Women	Total	2,877	84.2 ± 14.5 $	73.1 ± 12.1 $	11.0 ± 11.5
	Non-smokers	2,554	84.0 ± 14.6	73.0 ± 12.1	10.9 ± 11.6
	Ex-smokers	78	87.9 ± 14.7 #	75.9 ± 13.4	12.0 ± 11.3
	Continuous smokers	229	85.1 ± 13.6	73.4 ± 11.6	11.6 ± 10.8

a: For the definitions of smoking status, refer to Table 2.

b: The differences in the means of eGFR and the changes were tested between men and women and among the 3 groups of smoking status by a two-way analysis of variance. Subsequent paired comparisons were performed by a least square difference method.

$: Significant difference between men and women, #: Significant difference from non-smokers, ##: Significant difference from ex-smokers.