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Effect of coffee consumption on all-cause and total cancer mortality: findings from the JACC study

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Abstract Coffee consumption is known to be related to various health conditions. Recently, its antioxidant effects have been suggested to be associated with all-cause or cancer mortality by various cohort studies. However, there has been only one small Asian cohort study that has assessed this association. Thus, we tried to assess the association of coffee with all-cause and total cancer mortality by conducting a large-scale cohort study in Japan. A total of 97,753 Japanese men and women aged 40–79 years were followed for 16 years. Hazard ratios and 95% confidence intervals of all-cause and total cancer mortality in relation to coffee consumption were calculated from proportional-hazards regression models. A total of 19,532 deaths occurred during the follow-up period; 34.8% of these deaths were caused by cancer. The all-cause mortality risk decreased with increasing coffee consumption in both men and women, with a risk elevation at the highest coffee consumption level (≥4 cups/day) compared with the 2nd highest consumption level in women, although the number of subjects evaluated at this level was small. No association was found between coffee consumption and total cancer mortality among men, whereas a weak inverse association was found among women. The present cohort study among the Japanese population suggested that there are beneficial effects of coffee on all-cause mortality among both men and women. Furthermore, the results showed that coffee consumption might not be associated with an increased risk of total cancer mortality.

Keywords All-cause mortality · Coffee consumption · Cohort study · Total cancer mortality

Abbreviations

95% CI	95 percent confidence interval
BMI	Body mass index
HR	Hazard ratio
JACC Study	Japan Collaborative Cohort Study for Evaluation of Cancer Risk
MI	Myocardial infarction

The member list of the JACC study group are listed in the "Appendix".

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Introduction

Coffee contains a variety of biological compounds such as caffeine, caffeic acid, chlorogenic acid and diterpenes. Caffeine is known to stimulate tumors, inhibit insulin activity, increase blood pressure and increase homocysteine level, and all of these effects may be harmful to health. However, coffee also has beneficial effects in that it can prevent cancer and inflammation thorough its antioxidant activity. Moreover, it is suggested that caffeic acid inhibits DNA methylation, chlorogenic acid improves glucose

tolerance, diterpenes have anticarcinogenic properties, and various components of coffee may be associated with favorable effects on health [1, 2].

Studies examining the risk of coffee consumption and its association with all-cause mortality have produced inconsistent results. Some cohort studies have found an inverse association [3–5] with all-cause mortality, while other studies have found positive [6, 7] and U-shaped associations [8, 9]. Furthermore, data on coffee consumption in relation to total cancer mortality is sparse [3, 10, 11]. Several epidemiologic studies have examined coffee consumption and the risk of cancer by site, such as the liver [12, 13], kidney [14] and breast [15]. These studies mainly showed an inverse association with increasing consumption of coffee; however, the results of studies on other cancer sites have been inconsistent. There are several cohort studies from Asian countries that have confirmed the associations between coffee consumption and the risk of site-specific cancer [12, 13, 16, 17]; however, to the best of our knowledge, only one cohort analysis (with 2,855 subjects) has investigated the impact of coffee consumption as a risk factor for all-cause and total cancer mortality [3]. The objective of this study was to assess the association of coffee with all-cause and total cancer mortality using a large-scale cohort study in Japan with a follow-up period of 16 years.

Methods

Study subjects and data collection

The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC study) was started between 1988 and 1990, enrolling subjects living in 45 areas in Japan. Sampling methods and details of the JACC study have been described elsewhere [18]. Subjects were recruited mainly at the time of their health-checkup using a self-administered questionnaire and a response rate of 83% was obtained. We followed 110,792 subjects (46,465 men and 64,327 women), aged 40–79 years at baseline.

Information about coffee consumption and other lifestyles was obtained using the self-administered questionnaire. Subjects were grouped into four categories according to their daily coffee intake at baseline: those consuming less than 1 cup, 1 cup, 2–3 cups, or 4 or more cups. The question regarding coffee consumption was assessed previously by a validation study and a high agreement with 12-day weighted dietary records was reported (Spearman correlation: 0.81) [19]. A total of 97,753 individuals (40,672 men and 57,081 women) provided responses to the coffee consumption question, and were included in the analysis.

Follow-up

The date and cause of deaths were confirmed, with the permission of the Director-General of the Prime Minister's Office, up to the end of 2006 by death certificates, except in seven areas where follow-ups were discontinued at the end of 1999 or 2003. Individuals who moved away from the study area were treated as study dropouts because deaths after such moves could not be confirmed in our follow-up system. Our entire study design was approved by the Ethical Board of Nagoya University School of Medicine, where the central secretariat of the JACC study was located.

Analysis

The distribution of some socio-demographic factors was compared between different coffee consumption groups using ANOVA or the Mantel–Haenszel test adjusted for age category. Hazard ratios (HRs) were calculated by Cox's proportional hazard model adjusted for 5-year age groups separately by gender. In multivariate analyses, we further adjusted several factors which were known to be associated with all-cause mortality and/or coffee consumption–that is, smoking status (current smoker, former smoker, or never smoker), drinking status (current drinker, former drinker, or never drinker), daily walking duration (walking more than 1 h per day or not), sleep length (< 6.5 h, 6.5–8.4 h, ≥ 8.5 h), consumption of green-leafy vegetables (almost daily or not), green tea consumption (daily or not), perceived stress (yes or no), education (attended school up to 15–18, >18 years old), body mass index (BMI; < 18.5, 18.5–24.9, ≥ 25.0), marital status (having a spouse or not) or disease history (cancer, myocardial infarction, stroke, or none). Data for the above factors was self-reported. For all covariates, missing values were treated as an additional category in the model. The linear trend in mortality risk was assessed by treating the number of cups of coffee intake per day as an ordinal variable. Further analysis stratified by baseline age (40–59 years and 60–79 years) in addition to gender was also conducted. To evaluate reverse causation, the risk of mortality, excluding subjects with past history of cancer, MI or stroke, or deaths occurring every 2 years up to 8 years (half of median follow-up period) from baseline, was also estimated. All statistical analyses were performed using the Statistical Analysis System (SAS 9.1, Cary, NC) at the Aichi Medical University Computer Center.

Results

During the 16-year median follow-up period, 4,876 subjects (1,766 men and 3,110 women) dropped out of the

follow-up while 19,532 deaths (11,178 men and 8,354 women) occurred. Of these deaths, 34.8% (37.3%, men; 31.5%, women) were caused by cancer, followed by cardiovascular diseases (30.7%, total; 28.3%, men; 34.0%, women). Of men's cancer mortality, the five most common sites were the lung, stomach, liver, pancreas and colon (23.4, 19.3, 11.3, 5.7 and 5.7% of men's cancer deaths, respectively). Of women's cancer mortality, the five most common sites were the stomach, lung, pancreas, liver and colon (15.9, 11.9, 9.5, 9.3 and 9.2%, respectively).

As shown in Table 1, those who consumed high amounts of coffee were younger, more likely to be a smoker, educated, highly stressed, and less likely to drink green tea and have a past disease history; this applied to both men and women.

The HRs of all-cause and total cancer mortality based on coffee consumption are shown in Table 2. The risk of all-cause mortality decreased with increasing coffee consumption in both men and women; however, in women, there was a slight risk elevation at the highest coffee consumption level compared with the 2nd level. Multivariable-adjusted HRs of those who consumed 4 or more cups of coffee per day were 0.80 (95% CI: 0.68–0.95) in men and 0.89 (0.66–1.20) in women compared with those who consumed less than 1 cup of coffee per day. The risk of total cancer mortality did not show any association with coffee consumption in men, but was slightly reduced with increasing coffee consumption in women, with a significant decreasing trend. Table 3 shows the results of analysis performed separately for each age group. Although coffee drinkers were more apparent in the younger age group than in the older age group, the decreasing trend of all-cause mortality with increasing coffee consumption was apparent in either age group. For total cancer mortality risk, men of both age groups showed no association with coffee consumption. Women aged 40–59 at baseline showed a slightly non-significant decreasing trend with increased coffee consumption, whereas women aged 60–79 at baseline showed risk reduction in only the 2nd and 3rd levels.

The results of further analysis, with exclusion of those with past medical history or those who died early, to examine reverse causation for coffee consumption and mortality are shown in Table 4. Analysis performed excluding those with past history of cancer, MI or stroke showed no remarkable alteration of the results among both men and women. Sequent exclusion of deaths that occurred within 2–8 years from baseline did not change the association of coffee consumption with all-cause and total cancer mortality in men. In women, the all-cause mortality risk reductions of consumers of 1 cup or 2–3 cups of coffee per day were weakened and consumers with the highest consumption level showed HRs greater than 1.00 after the

Table 1 Baseline characteristics by coffee consumption

		Men					Women				
		<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	*P*	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	*P*
Participants	N	29,077	4,502	6,002	1,091		41,095	8,037	7,183	766	
Age (mean)	year	58.9	57.2	53.9	51.1	<0.0001	59.4	56.9	53.3	49.7	<0.0001
Current smoker	%	47.8	53.4	66.9	81.0	<0.0001	3.9	5.6	10.0	30.0	<0.0001
Current drinker	%	75.9	76.0	73.1	63.0	<0.0001	20.9	28.8	33.9	34.5	<0.0001
Walking 1 h/day ≤	%	50.8	47.6	46.4	44.7	<0.0001	51.5	50.4	51.2	50.6	0.27
Sleep 6.5–8.4 h/day	%	70.0	73.5	73.1	67.8	0.93	67.0	67.3	64.1	50.8	<0.0001
Eat green-leafy vegetables almost daily	%	26.2	29.0	26.4	23.0	<0.0001	30.6	34.4	32.8	32.3	<0.0001
Drink green tea daily	%	84.4	85.5	81.1	75.5	<0.0001	83.0	81.1	77.0	67.6	<0.0001
BMI (age-adjusted mean)	kg/m^2	22.5	22.5	22.4	22.2	<0.0001	22.8	22.7	22.7	22.3	<0.0001
Attend school up to 18 years old	%	12.2	19.8	21.2	25.0	<0.0001	7.1	10.9	12.5	14.6	<0.0001
Mentally stressed	%	19.7	24.4	30.5	38.3	<0.0001	18.4	20.4	25.9	29.9	<0.0001
Having a spouse	%	93.4	94.5	93.8	92.0	0.86	81.2	83.9	86.5	83.8	<0.01
Past history of cancer, MI or stroke	%	7.1	6.5	5.0	2.4	0.54	6.6	7.0	4.8	4.3	<0.0001

P values were calculated by ANOVA or Mantel–Haenszel test adjusted for 5-year age groups

BMI body mass index, *MI* myocardial infarction

Table 2 Hazard ratios for all-cause and total cancer mortality by coffee consumption

	Men					Women				
	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*
Person-years	413,510	61,914	83,801	15,545		613,607	111,465	100,872	10,944	
All causes										
Cases	8,989	1,035	1,004	150		7,098	738	474	44	
Age-adjusted HR (95% CI)	1.00	0.94 (0.88–1.00)	0.90 (0.84–0.96)	0.89 (0.76–1.05)	<.01	1.00	0.81 (0.75–0.87)	0.83 (0.76–0.91)	1.03 (0.7–1.38)	<.0001
Multivariable-adjusted HR (95% CI)	1.00	0.95 (0.89–1.01)	0.86 (0.81–0.93)	0.80 (0.68–0.95)	<.0001	1.00	0.82 (0.76–0.89)	0.83 (0.75–0.91)	0.89 (0.66–1.20)	<.0001
Total cancer										
Cases	3,246	408	436	76		2,142	278	191	17	
Age-adjusted HR (95% CI)	1.00	0.99 (0.90–1.10)	1.00 (0.90–1.11)	1.15 (0.91–1.44)	0.27	1.00	0.90 (0.79–1.02)	0.87 (0.75–1.02)	0.94 (0.58–1.51)	0.05
Multivariable-adjusted HR (95% CI)	1.00	1.02 (0.92–1.13)	0.97 (0.88–1.08)	1.06 (0.84–1.33)	0.74	1.00	0.89 (078–1.01)	0.85 (0.73–0.99)	0.81 (0.50–1.32)	0.01

Multivariable-adjusted HR; adjusted for age categories, smoking status, alcohol drinking, walking hours, sleep duration, body mass index, consumption of green-leafy vegetables, green tea consumption, education, stress, marital status, past history of cancer, myocardial infarction or stroke

exclusion of deaths, which occurred within 6–8 years from baseline. The inverse association of total cancer mortality in women was weakened and was no longer statistically significant after the exclusion of deaths that occurred within 6 years.

Discussion

The results suggest the beneficial effects of coffee on all-cause mortality in both men and women. For women, although the number of subjects in the highest level was small, the change of the RR by increasing level coffee consumption may suggest a U-shaped association. No association was found between coffee consumption and cancer mortality in men, whereas a weak inverse association was found in women. Sequent exclusion of deaths that occurred within 2–8 years from baseline did not alter the overall results; among women, however, the reduction in total cancer mortality risk was weakened and was not significant.

Previous cohort studies examined coffee consumption in relation to all-cause mortality, and the results were controversial. Early studies tended to find direct associations [6, 7]. However, recently, the occurrence of inflammation or coronary heart disease were found to be inversely associated with coffee consumption by some cohort studies [4, 9], and an inverse [3, 5] or U-shaped [8, 20] association between coffee consumption and all-cause mortality has also been reported. The results of our study detecting an inverse association among men and a U-shaped association among women were in line with these recent studies.

Some cohort studies have also examined the effect of coffee consumption on total cancer mortality. Most of these studies found no association [3, 4, 6, 9] while two studies found an inverse but not significant association [5, 11]. Our study also found no association among men and a weak inverse association among women between coffee consumption and total cancer mortality, even after exclusion of those with a past history of cancer, MI or stroke. Although the weak inverse association among women disappeared with the exclusion of deaths that occurred within 6 years after baseline, it suggests that at the least, there are no harmful effects of coffee on total cancer mortality.

Inconsistent results among men between the risks of all-cause mortality and total cancer mortality in our study must be due to an association with other causes of death, as cancer deaths accounted for about one-third of total mortality. We previously reported an inverse association between coffee consumption and the risk of total cardiovascular disease mortality among men [21]. In addition, the incidence of diabetes mellitus was found to be lower with increasing coffee consumption [22]. Some recent cohort

Table 3 Hazard ratios for all-cause and total cancer mortality by coffee consumption stratified by age group at baseline

	Men					Women				
	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*
Person-years										
Aged 40–59 years at baseline	231,833	39,599	62,216	12,682		327,339	70,973	76,533	9,599	
Aged 60–79 years at baseline	181,677	22,314	21,585	2,863		286,268	40,493	24,340	1,345	
All causes										
Aged 40–59 years at baseline										
Cases	2,097	250	366	63		1,204	214	177	25	
Age-adjusted HR (95% CI)	1.00	0.78 (0.68–0.89)	0.83 (0.74–0.93)	0.79 (0.61–1.01)	<.001	1.00	0.94 (0.82–1.09)	0.82 (0.70–0.96)	1.04 (0.70–1.56)	0.07
Multivariable-adjusted HR (95% CI)	1.00	0.80 (0.70–0.91)	0.80 (0.71–0.90)	0.69 (0.54–0.89)	<.0001	1.00	0.94 (0.81–1.10)	0.79 (0.67–0.93)	0.83 (0.56–1.25)	0.01
Aged 60–79 years at baseline										
Cases	6,892	785	638	87		5,894	524	297	19	
Age-adjusted HR (95% CI)	1.00	1.00 (0.93–1.08)	0.93 (0.86–1.01)	0.96 (0.78–1.19)	0.35	1.00	0.77 (0.70–0.84)	0.85 (0.75–0.95)	1.02 (0.65–1.60)	<.0001
Multivariable-adjusted HR (95% CI)	1.00	1.01 (0.93–1.08)	0.90 (0.83–0.98)	0.89 (0.72–1.10)	0.06	1.00	0.79 (0.72–0.86)	0.86 (0.76–0.97)	0.88 (0.56–1.39)	<.0001
Total cancer										
Aged 40–59 years at baseline										
Cases	943	107	175	43		584	111	98	10	
Age-adjusted HR (95% CI)	1.00	0.75 (0.61–0.92)	0.91 (0.77–1.07)	1.25 (0.92–1.70)	0.96	1.00	1.00 (0.81–1.22)	0.91 (0.73–1.13)	0.84 (0.45–1.57)	0.35
Multivariable-adjusted HR (95% CI)	1.00	0.77 (0.63–0.95)	0.90 (0.76–1.06)	1.16 (0.84–1.59)	0.77	1.00	0.99 (0.80–1.22)	0.89 (0.71–1.11)	0.71 (0.38–1.35)	0.17
Aged 60–79 years at baseline										
Cases	2,303	301	261	33		1,558	167	93	7	
Age-adjusted HR (95% CI)	1.00	1.11 (0.99–1.26)	1.05 (0.93–1.20)	1.01 (0.72–1.43)	0.14	1.00	0.84 (0.71–0.98)	0.84 (0.68–1.04)	1.17 (0.56–2.47)	0.05
Multivariable-adjusted HR (95% CI)	1.00	1.14 (1.01–1.29)	1.03 (0.90–1.17)	0.94 (0.67–1.33)	0.40	1.00	0.83 (0.70–0.98)	0.82 (0.66–1.02)	1.00 (0.47–2.12)	0.03

Multivariable-adjusted HR; adjusted for age categories, smoking status, alcohol drinking, walking hours, sleep duration, body mass index, consumption of green-leafy vegetables, green tea consumption, education, stress, marital status, past history of cancer, myocardial infarction or stroke

Table 4 Multivariable-adjusted hazard ratios of all-cause and total cancer mortality by coffee consumption with exclusion of subjects with past disease history or early deaths

	Men					Women				
	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*
Person-years										
Past history of cancer, MI or stroke excluded	394,503	59,430	81,228	15,280		586,564	106,547	97,900	10,586	
Death within first 2 years excluded	412,686	61,811	83,653	15,516		612,881	111,349	100,765	10,934	
Death within first 4 years excluded	409,867	61,373	83,212	15,434		610,371	111,043	100,436	10,907	
Death within first 6 years excluded	404,285	60,619	82,333	15,246		605,595	110,290	99,993	10,820	
Death within first 8 years excluded	395,733	59,535	80,980	15,077		597,756	109,188	99,125	10,722	
All cause										
Past history of cancer, MI or stroke excluded										
Cases	8,102	933	910	142		6,413	675	434	43	
HR (95% CI)	1.00	0.94 (0.88–1.01)	0.85 (0.79–0.91)	0.79 (0.67–0.94)	<.0001	1.00	0.83 (0.77–0.91)	0.83 (0.75–0.92)	0.94 (0.69–1.27)	<.0001
Death within first 2 years excluded										
Cases	8,382	972	940	143		6,727	689	446	43	
HR (95% CI)	1.00	0.96 (0.90–1.03)	0.87 (0.82–0.94)	0.83 (0.70–0.98)	<.001	1.00	0.82 (0.75–0.89)	0.83 (0.75–0.92)	0.92 (0.68–1.25)	<.0001
Death within first 4 years excluded										
Cases	7,606	864	848	130		6,183	637	405	42	
HR (95% CI)	1.00	0.96 (0.89–1.03)	0.87 (0.81–0.94)	0.82 (0.69–0.98)	<.001	1.00	0.84 (0.77–0.91)	0.84 (0.75–0.93)	1.00 (0.73–1.36)	<.001
Death within first 6 years excluded										
Cases	6,653	744	729	106		5,512	555	359	37	
HR (95% CI)	1.00	0.96 (0.89–1.04)	0.88 (0.81–0.95)	0.77 (0.64–0.94)	<.001	1.00	0.84 (0.77–0.92)	0.85 (0.76–0.95)	1.00 (0.72–1.39)	<.01
Death within first 8 years excluded										
Cases	5,568	606	594	93		4,673	450	296	31	
HR (95% CI)	1.00	0.97 (0.89–1.06)	0.88 (0.80–0.96)	0.82 (0.67–1.01)	<.01	1.00	0.86 (0.78–0.95)	0.88 (0.78–0.99)	1.04 (0.73–1.49)	0.03
Cancer										
Past history of cancer, MI or stroke excluded										
Cases	3,039	378	401	74		1,983	254	177	17	
HR (95% CI)	1.00	1.01 (0.90–1.12)	0.94 (0.85–1.05)	1.06 (0.84–1.35)	0.98	1.00	0.88 (0.77–1.01)	0.84 (0.72–0.99)	0.89 (0.55–1.44)	0.02
Death within first 2 years excluded										
Cases	3,034	382	411	75		2,001	258	183	17	
HR (95% CI)	1.00	1.03 (0.92–1.15)	0.99 (0.88–1.10)	1.12 (0.89–1.42)	0.38	1.00	0.88 (0.77–1.01)	0.87 (0.74–1.02)	0.86 (0.53–1.40)	0.04
Death within first 4 years excluded										
Cases	2,728	335	373	69		1,798	233	161	16	
HR (95% CI)	1.00	1.01 (0.90–1.13)	0.99 (0.88–1.11)	1.13 (0.88–1.44)	0.34	1.00	0.90 (0.78–1.03)	0.85 (0.72–1.01)	0.90 (0.55–1.49)	0.05
Death within first 6 years excluded										
Cases	2,335	290	321	49		1,552	196	142	14	
HR (95% CI)	1.00	1.04 (0.91–1.17)	1.00 (0.88–1.13)	0.93 (0.70–1.24)	0.93	1.00	0.90 (0.77–1.05)	0.89 (0.74–1.06)	0.92 (0.54–1.57)	0.14

Table 4 continued

	Men					Women				
	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*	<1 cup/day	1 cup/day	2–3 cups/day	4+ cups/day	Trend *P*
Death within first 8 years excluded										
Cases	1,922	235	257	42		1,271	154	116	12	
HR (95% CI)	1.00	1.05 (0.91–1.21)	0.99 (0.86–1.13)	0.97 (0.71–1.32)	0.96	1.00	0.91 (0.77–1.08)	0.92 (0.76–1.13)	1.00 (0.56–1.79)	0.46

HR adjusted for age categories, smoking status, alcohol drinking, walking hours, sleep duration, body mass index, consumption of green-leafy vegetables, green tea consumption, education, stress, marital status, past history of cancer, myocardial infarction or stroke

studies examined the risk of coffee consumption on cardiovascular diseases, which was also an inverse association [4, 9]. Cardiovascular deaths accounted for 28.3% of men's deaths in our study; thus, this may be the main reason that an inverse association with all-cause mortality occurs, in spite of no association with total cancer mortality among men.

Recently, coffee has been found to be one of the major sources of antioxidants in the diet [23] and has beneficial effects on inflammation [9]. It has been found that plasma antioxidants increased [24] and biomarkers of oxidative stress decreased [25] after coffee intake. Prolonged inflammation may contribute not only to atherosclerosis and ischemic heart diseases, but also to cancer. The beneficial effects of coffee on all-cause mortality and total cancer mortality might be understandable from this point of view. On the other hand, there is a possibility that possible subclinical diseases lead to a reduction in coffee consumption. In fact, those in the lowest coffee consumption group had more past disease history than those who consumed 1 cup of coffee a day or more in our study. This poorer health condition might be linked to a risk elevation among non-coffee drinkers. However, analyses to examine the reverse causation with sequent exclusion of deaths that occurred within 2–8 years from baseline did not alter the relationship between coffee consumption and all-cause mortality, suggesting that the difference in health at baseline was not the only explanation of the associations that were found.

There are two methodological limitations in this study. First, we obtained information only at baseline. Moreover, coffee consumption was estimated from self-report. Thus, some measurement errors at baseline were inevitable. Second, detailed data on coffee consumption, such as choice of caffeinated or decaffeinated and methods of brewing, were not collected. The methods of coffee preparation and habits of coffee drinking may change considerably with time and vary geographically. Therefore, other cohort studies with detailed coffee consumption data are required.

In conclusion, the present cohort study suggested the beneficial effects of coffee consumption on all-cause mortality among both men and women. In addition, the results indicated no association between coffee consumption and total cancer mortality among men and a weak inverse association among women, suggesting that no harmful effects of coffee on total cancer mortality.

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Conflict of interest The authors declare that there are no conflicts of interest.

Appendix: Member list of the JACC study group

The present members of the JACC Study Group who co-authored this paper together with their affiliations are as follows: Dr. Akiko Tamakoshi (present chairperson of the study group), Aichi Medical University School of Medicine; Drs. Mitsuru Mori & Fumio Sakauchi, Sapporo Medical University School of Medicine; Dr. Yutaka Motohashi, Akita University School of Medicine; Dr. Ichiro Tsuji, Tohoku University Graduate School of Medicine; Dr. Yosikazu Nakamura, Jichi Medical School; Dr. Hiroyasu Iso, Osaka University School of Medicine; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Michiko Kurosawa, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr. Naohito Tanabe, Niigata University School of Medicine; Dr. Koji Tamakoshi, Nagoya University Graduate School of Health Science; Dr. Kenji Wakai, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, National Institute of Health and Nutrition; Dr. Koji Suzuki, Fujita Health University School of Health Sciences; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Yasuhiko Wada, Faculty of Human Life and Environmental Science, Kochi Women's University; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Dr. Yoshiyuki Watanabe, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Kotaro Ozasa, Radiation Effects Research Foundation; Dr. Tsuneharu Miki, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Chigusa Date, School of Human Science and Environment, University of Hyogo; Dr. Kiyomi Sakata, Iwate Medical University; Dr. Yoichi Kurozawa, Tottori University Faculty of Medicine; Drs. Takesumi Yoshimura & Yoshihisa Fujino, University of Occupational and Environmental Health; Dr. Akira Shibata, Kyushu University Institute of Health Science; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; and Dr. Hideo Shio, Moriyama Municipal Hospital.

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Number of children and all-cause mortality risk: results from the Japan Collaborative Cohort Study

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Background: The mean total birth rate of the world had been gradually decreasing, with the rate in Japan now at its lowest level internationally. From a public health perspective, it is important to determine the impact of the number of children on all-cause mortality. **Methods:** A total of 96 311 individuals from the Japan Collaborative Cohort Study were followed from 1988–90 for an average of 14.4 years. Hazard ratios (HRs) with a 95% confidence interval were calculated from proportional hazard models to estimate the risk of all-cause mortality according to the number of children. **Results:** As of 2006, a total of 18 807 deaths had occurred. Both childless men and women showed higher all-cause mortality risks than those with two children (HR: 1.17 in men and 1.29 in women). Those with one child also showed higher risks (1.13 and 1.16, respectively). Having four or more children among men and five or more children among women also posed a risk (1.16 in men with four children and 1.22 in women with five or more children), showing a U-shaped association between the number of children and all-cause mortality risk. The risk of having only one child seemed evident with the decrease in age among both men and women, while the risk of having many children was apparent with the increase in age. **Conclusion:** We found a U-shaped association between the number of children and all-cause mortality among both men and women, with the lowest risk among those with two children.

Keywords: childless, cohort study, mortality, number of children

Introduction

Because of the possible biological effects of reproductive history on mortality, many studies have examined the relationship between the number of children and all-cause mortality among women. However, only a few studies have focused on both men and women.[1–5] Norwegian census data covering 14.5 million person-years revealed that, compared to parents with two children, those who were childless or had only one child showed a significantly elevated risks of all-cause mortality, whereas those with three or more children showed significantly decreased risks in both men and women.[2] In rural Bangladesh, where the mean number of parity was seven live births at the time of the study, Hurt *et al.*[3] reported no association between parity and long-term mortality among women; however, there was a small but significant decrease in mortality among men with an increasing number of live births. A cohort study of those born between 1880 and 1929 found that parous women had a significantly poorer survival rate than nulliparous women (especially among those born earlier), but not among men.[1] These inconsistent findings among different periods, countries and cultures suggest that some social factors might be related to the association between the numbers of children and all-cause mortality risks.

According to the Population Database announced by the United Nations,[6] the mean total fertility rate of the world had been gradually decreasing (4.92 in 1950–55, 4.32 in 1970–75 and 2.67 in 2000–05), and is predicted to fall to 2.02 by 2045–50. In Japan, birth rates have been dramatically decreasing in recent years, with the total fertility rate now at its lowest level internationally (1.37 in 2008). Since such a dramatic decrease in the fertility rate is occurring worldwide, from a public health perspective, it is important to determine the impact of the number of children on all-cause mortality among both men and women in Japan, a country with one of the greatest decreases in this regard. In this study, using a large-scale cohort study initiated in Japan ~20 years ago, we investigated the relationship between the number of children and all-cause mortality among Japanese adults.

Methods

Subjects and data collection

The Japan Collaborative Cohort Study (JACC Study) was conducted from 1988 to 1990, enrolling healthy subjects living in 45 areas of Japan, and collecting baseline data using a self-administered questionnaire. Sampling methods and detailed protocols have been published elsewhere.[7,8] A total of 110 792 subjects (46 465 males and 64 327 females) aged 40–79 years participated in the study.

Information about their children was obtained by self-administered questionnaires. Subjects were grouped into six categories according to the numbers of children—those who were childless, and those with 1, 2, 3, 4 and ≥5 children. A total of 96 311 individuals (40 073 men and 56 238 women) who provided valid responses were regarded as eligible for the analysis.

Our entire study design was approved by the Ethical Board of the Nagoya University School of Medicine, where the central secretariat of the JACC Study was located.

Follow-up

The causes and dates of death among the subjects were identified by reviewing all death certificates in each area with the permission of the Director-General of the Prime Minister's Office (Ministry of Internal Affairs and Communications). Those who moved out of a study area were treated as censored, because deaths after such moves could not be confirmed by our follow-up system. We followed the subjects until the end of 2006, except in eight areas where follow-ups were discontinued at the end of 1999 or 2003.

Analysis

The distribution of some socio-demographic factors that were known to be risk factors for all-cause mortality among groups of numbers of children were compared using an analysis of variance or the Mantel–Haenszel test adjusted for age. Hazard ratios (HRs) and 95% confidence interval (CI) were calculated using the Cox's proportional hazard model adjusted for 5-year age groups stratified by gender. We drew a graph of the log(−log(survival)) vs. log of survival time to check the proportional hazard assumption between the number of children. The lines were approximately parallel, confirming that the assumption was suitable. In multivariate analyses, we further adjusted several factors associated with all-cause mortality and/or number of children, as well as the subjects' residential area grouped into seven by geographic location—that is, smoking status (current smoker, quitter or never-smoker), drinking status (current drinker, quitter or never-drinker), daily walking length (walking >1 h per day or not), sleep length (<6.5 h, 6.5–8.4 h, 8.5 h or longer), consumption of green-leafy vegetables (almost daily or not), perceived stress (yes or no), education (attended school up to 15-years old, 18-years old or older), body mass index (BMI < 18.5, 18.5–24.9, 25.0≤), occupational status (employed, self-employed or not), marital status (having a spouse or not), disease history (of cancer, cardiovascular disease or stroke or none), all of these data were obtained from self-administered questionnaires. For all covariates, missing values were treated as an additional category in the model. Further analysis stratified by baseline age (10 years each) in addition to gender was also conducted. All statistical analyses were performed using the Statistical Analysis System (SAS 9.1, Cary, NC, USA) at the Aichi Medical University Computer Center.

Results

During the average follow-up period of 14.4 years, a total of 4733 movements (1732 men and 3001 women) and 18 807 deaths occurred (10 849 men and 7958 women). Total cancer deaths accounted for 37.1% in men and 31.3% in women, with circulatory system deaths accounting for 28.6% and 34.6%, respectively.

A total of 1369 (3.4%) men and 2026 (3.6%) women were childless at baseline. The largest group was composed of those with two children among both men (41.6%) and women (38.2%), followed by those with three children (32.7% in men and 30.8% in women) (table 1). Compared to those with one child, childless subjects were older, less likely to have a spouse, to walk, to eat green-leafy vegetables, or to show a healthy BMI (18.5–24.9), and were more likely to suffer from mental stress, to have a history of cancer, cardiovascular disease or stroke, less likely to be employed among both men and women, and more likely to be non-drinkers among men. Among subjects with children, those with two children were the youngest (54.9 years among men and 54.1 years among women), with the mean age increasing according to the increasing number of children, and the oldest mean age found among those with five or more children (69.9 among men and 69.7 among women). The proportion of those having a spouse, sleeping 6.5–8.4 h per night, with a BMI of 18.5–24.9, being highly educated, without a history of cancer, cardiovascular disease or stroke and employed showed a similar pattern, reaching its highest among those with two children and its lowest among those with five or more in both men and women.

The all-cause mortality risks according to the number of children are shown in table 2. Compared to those with two children, the childless group and that with one child showed a slightly higher all-cause mortality risk—1.31 (95% CI 1.19–1.45) in men and 1.37 (1.24–1.53) in childless women and 1.18 (1.10–1.28) in men and 1.21 (1.10–1.32) in women with one child; though those values decreased slightly after adjusting for potential risk factors, they still remained at statistically significant levels. Men with three children showed no risk elevation, whereas men with four or more children were associated with elevated risk. A similar trend was observed among women, although the protective effect of having children was more apparent than in men, and only those with five or more children showed an excessive risk.

With stratification by age group at the baseline, different distribution patterns of the number of children were observed. Among those aged 40–49 years, most had two children (51.5% men and 52.6% women). However, the proportion was diminished according to increasing age, and among those aged 70–79 years, these values declined to 16.1 and 13.1%, respectively. In contrast, those with five or more children were rare among the younger generation (0.6 and 0.6% among men and women aged 40–49 years) increasing according to increasing age and reaching 20.3 and 31.5% among those aged 70–79 years. As shown in table 3, among both men and women, the risk of having only one child became apparent with age reduction, though the risk among men aged 40–49 years was lower than that in other age groups. Moreover, all-cause mortality risks of the childless were lower than those with one child among them: 1.19 (0.78–1.81) and 1.05 (0.79–1.40) among men aged 40–49, 1.36 (0.79–2.32) and 1.53 (1.13–2.09) among women aged 40–49 years and 0.89 (0.63–1.25) and 1.29 (1.07–1.57) among women aged 50–59, respectively, while, the values were 1.13 (0.95–1.34) and 1.08 (0.92–1.27) among men aged 70–79 and 1.36 (1.15–1.62) and 1.09 (0.93–1.27) among women aged 70–79, respectively. In contrast, the risk of having many children was evident from the age increment. Especially among women, null associations

Table 1 Distribution of some demographic factors according to the number of children: baseline of the JACC Study 1988–90, Japan

	Men						Women					
	0	1	2	3	4	≥5	0	1	2	3	4	≥5
Number	1369	3159	16 656	13 110	3875	1904	2026	4842	21 508	17 348	6228	4286
Age (years)	58.1 ± 11.0	56.5 ± 9.9	54.9 ± 8.9	57.8 ± 9.9	64.9 ± 9.6	69.9 ± 8.0***	60.6 ± 10.1	57.6 ± 9.8	54.1 ± 8.7	57.1 ± 9.5	63.9 ± 8.8	69.7 ± 7.3***
Having a spouse	668 (48.8)	2632 (83.3)	14 540 (87.3)	11 208 (85.5)	3052 (78.8)	1391 (73.1)***	916 (45.2)	3254 (67.2)	17 059 (79.3)	13 234 (76.3)	4069 (65.3)	2397 (55.9)***
Never-smoker	256 (18.7)	567 (17.9)	3340 (20.1)	2585 (19.7)	684 (17.7)	372 (19.5)*	1522 (75.1)	3712 (76.7)	17 690 (82.2)	14 470 (83.4)	5013 (80.5)	3384 (79.0)***
Non-drinker	336 (24.5)	606 (19.2)	2951 (17.7)	2162 (16.5)	742 (19.1)	426 (22.4)***	1345 (66.4)	3236 (66.8)	14 665 (68.2)	12 111 (69.8)	4365 (70.1)	3068 (71.6)*
Walking ≥ 1 h day^{-1}	358 (26.2)	1225 (38.8)	6935 (41.6)	5535 (42.2)	1646 (42.5)	771 (40.5)***	561 (27.7)	1953 (40.3)	9215 (42.8)	7652(44.1)	2740 (44.0)	1704 (39.8)***
Sleep 6.5–8.4 h per night	840 (61.4)	2198 (69.6)	11 906 (71.5)	9088 (69.3)	2402 (62.0)	1054 (55.4)*	1234 (60.9)	3101 (64.0)	14 090 (65.5)	11 154 (64.3)	3732 (59.9)	2353 (54.9)***
Eating green-leafy vegetables almost daily	215 (15.7)	771 (24.4)	4259 (25.6)	3501 (26.7)	1132 (29.2)	541 (28.4)***	469 (23.1)	1497 (30.9)	6665 (31.0)	5278 (30.4)	1995 (32.0)	1275 (29.7)
BMI 18.5–24.9	922 (67.3)	2278 (72.1)	12 339 (74.1)	9559 (72.9)	2731 (70.5)	1284 (67.4)***	1204 (59.4)	3227 (66.6)	15 091 (70.2)	11 750 (67.7)	3907 (62.7)	2426 (56.6)***
College or higher education	180 (13.1)	459 (14.5)	2554 (15.3)	1767 (13.5)	473 (12.2)	184 (9.7)***	200 (9.9)	430 (8.9)	2073 (9.6)	1302 (7.5)	316 (5.1)	139 (3.2)***
Low mental stress	581 (42.4)	1898 (60.1)	9910 (59.5)	7731 (59.0)	2387 (61.6)	1124 (59.0)	954 (47.1)	2983 (61.6)	13 642 (63.4)	10 918 (62.9)	3954 (63.5)	2607 (60.8)***
Without a history of cancer, cardiovascular disease or stroke	869 (63.5)	2326 (73.6)	12 774 (76.7)	9834 (75.0)	2651 (68.4)	1247 (65.5)	1366 (67.4)	3437 (71.0)	16 192 (75.3)	12 986 (74.9)	4383 (70.4)	2899 (67.6)***
Employed	410 (29.9)	1259 (39.9)	7143 (42.9)	4028 (30.7)	617 (15.9)	176 (9.2)***	335 (16.5)	1079 (22.3)	5907 (27.5)	3516 (20.3)	625 (10.0)	226 (5.3)

Data represent number of individuals (percentage in parentheses) or mean ± standard error for continuous variables
*P<0.05, **P<0.01, ***P<0.001 performed by ANOVA for age or performed by Mantel–Haenszel test adjusted for age (categorical data)

Table 2 All-cause mortality risks according to the number of children: the JACC Study 1988–2006, Japan

	Men						Women					
	0	1	2	3	4	≥5	0	1	2	3	4	≥5
Total												
Person-years	19 118	44 364	244 999	188 060	48 944	21 202	29 334	68 648	323 455	259 853	88 158	55 328
Cases	474	799	3205	3449	1765	1157	469	685	1783	2032	1342	1647
Age-adjusted HR												
HR (95% CI)	1.31 (1.19–1.45)	1.18 (1.10–1.28)	1.00	1.05 (1.00–1.10)	1.20 (1.13–1.28)	1.32 (1.23–1.42)	1.37 (1.24–1.53)	1.21 (1.10–1.32)	1.00	1.02 (0.96–1.09)	1.07 (0.99–1.15)	1.24 (1.15–1.34)
Multi-adjusted HR												
HR (95% CI)	1.17 (1.06–1.30)	1.13 (1.04–1.22)	1.00	1.04 (0.99–1.09)	1.16 (1.09–1.24)	1.25 (1.16–1.35)	1.29 (1.16–1.44)	1.16 (1.06–1.27)	1.00	1.03 (0.97–1.10)	1.06 (0.99–1.15)	1.22 (1.13–1.32)

Multi-adjusted HRs were adjusted for age, residential area group, marital status, smoking status, alcohol consumption status, walking hours, sleeping hours, consuming green-leafy vegetables, BMI, education, mental stress, disease history and employment status

Table 3 All-cause mortality risks stratified by age categories according to the number of children: the JACC Study 1988–2006, Japan

	Men						Women					
	0	1	2	3	4	≥5	0	1	2	3	4	≥5
Aged 40–49 at baseline (years)												
Person-years	5435	13 666	81 418	50 963	5578	936	5417	17 709	112 303	69 195	8063	1292
Cases	30	58	304	197	26	4	16	53	192	127	10	2
HR (95% CI)	1.19 (0.78–1.81)	1.05 (0.79–1.40)	1.00	1.02 (0.85–1.22)	1.20 (0.80–1.80)	0.96 (0.35–2.58)	1.36 (0.79–2.32)	1.53 (1.13–2.09)	1.00	1.05 (0.84–1.32)	0.67 (0.35–1.27)	0.78 (0.19–3.13)
Aged 50–59 at baseline (years)												
Person-years	6108	15 188	93 474	58 545	8320	2136	8833	24426	131 009	86 419	16 678	4433
Cases	89	198	955	638	120	24	38	136	518	361	72	21
HR (95% CI)	1.20 (0.95–1.51)	1.24 (1.06–1.45)	1.00	1.07 (0.97–1.19)	1.34 (1.10–1.62)	0.91 (0.60–1.36)	0.89 (0.63–1.25)	1.29 (1.07–1.57)	1.00	1.04 (0.91–1.19)	1.02 (0.80–1.32)	1.01 (0.65–1.58)
Aged 60–69 at baseline (years)												
Person-years	4978	12 147	60 022	60 544	20 368	6635	9815	19 175	67 876	85 256	42 622	20 312
Cases	165	328	1398	1564	643	249	165	237	694	905	530	317
HR (95% CI)	1.17 (0.99–1.39)	1.09 (0.97–1.23)	1.00	1.02 (0.95–1.10)	1.18 (1.08–1.30)	1.35 (1.18–1.55)	1.31 (1.09–1.56)	1.11 (0.95–1.28)	1.00	0.98 (0.89–1.08)	1.05 (0.94–1.18)	1.24 (1.08–1.42)
Aged 70–79 at baseline (years)												
Person-years	2597	3364	10 085	18 008	14 678	11 495	5268	7338	12 268	18 984	20 795	29 291
Cases	190	215	548	1050	976	880	250	259	379	639	730	1307
HR (95% CI)	1.13 (0.95–1.34)	1.08 (0.92–1.27)	1.00	1.04 (0.94–1.16)	1.11 (1.00–1.24)	1.22 (1.09–1.37)	1.36 (1.15–1.62)	1.09 (0.93–1.27)	1.00	1.12 (0.98–1.27)	1.09 (0.96–1.23)	1.23 (1.09–1.38)

Multi-adjusted HRs were adjusted for age, residential area group, marital status, smoking status, alcohol consumption status, walking hours, sleeping hours, consuming green-leafy vegetables, BMI, education, mental stress, disease history and employment status

between having many children and all-cause mortality were found in those aged 40–49 and 50–59 years; however, in women aged 60–69 and 70–79 years, the risks of having five or more children were 1.24 (1.08–1.42) and 1.23 (1.09–1.38), respectively, compared with those having two children.

Discussion

Using the large data set of a population-based cohort study, we found that both men and women with no or only one child were at a significantly higher risk of all-cause mortality compared with those with two children. Additionally, men with four or more children and women with five or more children also had a significantly high risk, showing the U-shaped association with the number of children, with its lowest risk at having two children. The risk of having only one child seemed evident with the decrease in age among both men and women, while the risk of having many children was particularly apparent with the increase in age.

Results of studies examining the relationship between the number of children and all-cause mortality varied. A study conducted in Bangladesh showing that survival for both sexes was greatly enhanced by an increasing number of surviving children, regardless of parity or other social factors, indicates the social and economic advantages of having children in developing countries.[3] A study from Norway—where child allowances are relatively large and many 'family-friendly' policies exist—also found no high-parity disadvantage, suggesting that there are various health benefits of having several children in such an environment that may outweigh the costs.[2] Meanwhile, an Israeli study based on census data recently found a U-shaped association between the number of children and all-cause mortality, with the lowest risk being evident among those with 3–4 children for both sexes.[4] There were only a few studies examining the relationship among both men and women; however, among women, some other studies have also reported a U-shaped association with women with two children experiencing the lowest risk, as in our present study.[9–11] Such inconsistent results can be expected, since the impact of having children on the subjects' health might be considerably modified by their surrounding social factors.

The existence of children may provide subjects with parental roles, family obligations and social networks through childbearing.[5] These social factors may be related to healthier behavior,[2] may increase well-being,[12] and may consequently affect subsequent survival. Moreover, adult children may provide support to their elderly parents, mediate social services and monitor their health behavior.[5] Such conditions may offer a positive effect of having children, and subsequently lower the all-cause mortality risk. Although large family size usually involves parents in physical activity, it might also lead to excessive physical and mental stress.[13] Moreover, those with many children tended to start their parenthood earlier, with early parenthood known to be associated with poor health.[2] These social factors might partly explain the disadvantage in all-cause mortality risk of having many children. On the other hand, among the childless, especially among the elderly, the absence of social support from children might lead to an increase in all-cause mortality risk.[5]

The disadvantage of having no children was more apparent in women than in men, while the disadvantage of having many children was less in women than in men. Among women, the association between the number of children and all-cause mortality may also involve a biological mechanism, such as hormonal protection.[14,15] A prospective study of Norwegian women found inverse associations with parity and cancers of the breast, uterus and ovary.[16] Using the Swedish registry

system, Mogren *et al.*[17] also revealed that multiparity was a protective factor for all gynecological cancers, including cervical and breast cancers. We also previously demonstrated the protective effects of having children against the development of colon cancer among women.[15] Such effects on hormone-related diseases might be related to the biological changes initiated by conception and that lead to parity and breastfeeding, and might explain the somewhat different impact between men and women of having many children. On the other hand, parous women had a higher mortality rate from diabetes mellitus, ischemic heart disease and cerebrovascular disease than nulliparous women.[18] Parity was found to increase the risk of atherosclerosis through its effect on lower high-density lipoprotein levels and higher glucose/insulin ratios.[19] The negative effect on cardiovascular disease may explain the elevated mortality risk in women with five or more children.

Impacts on all-cause mortality differed with the number of children in the different age categories in our results, particularly among women. All-cause mortality is comprised of a combination of death from cancer, cardiovascular disease and other diseases, and the combination of causes of death differs with age. In fact, in our data set, the proportion of cancer deaths diminished with age (from 42.7 and 52.9% of total deaths in men and women aged 40–49 years to 26.9 and 21.1% in those aged 70–79 years, respectively), while cardiovascular deaths increased (from 19.8 and 19.5% up to 32.7 and 40.9%, respectively). This different composition might partially explain the differences in risk among different age categories. Some previous studies have examined the association between the number of children and all-cause mortality risks by age categories. The younger the women, the greater was the all-cause mortality risk of being childless, as was found in England, Wales, Austria[9] and Norway.[2] One study reported that the risk of all-cause mortality with many children was higher in those aged 50 years and older than in those aged <50 years.[16] Grundy and Tomassini, however, found the opposite result (all-cause mortality risk with five or more children was higher in a younger cohort than in the elderly).[11] The reason for such an inconsistent finding was unclear, but because of the availability of contraception and legal abortion, having many children might be based on one's choice among more recent generations than among earlier ones.[2] In fact, our results showed that there was a lower risk of many children among the middle-aged group than in the elderly group. In addition, from our study, the background of those without children and those with one child might be differ in the middle-aged and elderly group, though the middle-aged involved fewer events, given that they were less likely to die than the elderly. Mortality risks among those with one child were higher than those without children among middle-aged women and men except men aged 40–49 years unlikely to elder groups. This might be because the childless were better educated, more financially secure, and might feel more in control of their lives than women with children do nowadays.[20] At least we could say that not only the different causes of death in each age category but also different social and environmental factors between elderly and middle-aged women's cohorts might affect the association between the number of children and all-cause mortality risks.

The strong points of our study are as follows: (i) a large-scale cohort with more than 90 000 subjects from all over Japan; (ii) a long follow-up period of ~14.4 years; (iii) multiple lifestyle variables collected at baseline and (iv) adjusting as much as possible for potential confounders. Such advantages allowed us to separately estimate the association between the number of children and all-cause mortality by baseline age, while adjusting for various factors.

One limitation in this study was that the underlying reasons for being childless remain unknown. One possible reason for the high mortality-risk of childless women might be the poor health of our subjects. Childless subjects in our study were physically inactive, had an unhealthy BMI (<18.5 or ≤25), suffered from a high level of mental stress, and had a history of cancer, cardiovascular disease or stroke, compared to their counterparts with children. This suggests the possibility that the elevated risk among the childless group was due to neither the result of a reduced social network nor to other possible mechanisms, but simply to poor health. According to the statistics, the total fertility rate has rapidly decreased in Japan. It was around 2.0 from 1957 to 1974, but has fallen to below 1.50 since 1993.[21] The proportion of childless Japanese couples after 15–19 continuous years of marriage increased from 3.0% in 1977 to 5.6% in 2005.[22] Moreover, not only in Japan but also globally, fertility rates are declining.[6] Even if the main reason for being childless in the past was poor health, it may now be changing, so that assumptions cannot easily be made about the mortality risk patterns of childless men and women. Another limitation was that, although we had adjusted for potential confounding factors such as smoking status and physical activity, there was still a possibility that other factors (particularly social or psychological factors) might have confounded our findings. A large-scale cohort study with information on such social factors will be required to effectively investigate the true relationship between the number of children and mortality.

In conclusion, we found a U-shaped association between the number of children and all-cause mortality among both men and women, with the lowest risk among those with two children. Our main finding was that having no children or only one child may lead to an elevated risk of total mortality in view of the decrease in total future fertility rates, while emphasizing that a high risk in men and women with many children may not play an important role in the decline of fertility rates.

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Conflicts of interest: None declared.

Key points

- The mean total birth rate worldwide has been gradually decreasing, with the rate in Japan now at its lowest level internationally.
- We found that being childless or having only one child may lead to an elevated risk of total mortality.
- Men with four or more children and women with five or more children also showed a high risk of all-cause mortality.

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Appendix 1

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Induction of glandular stomach cancers in *Helicobacter pylori*-infected Mongolian Gerbils by 1-nitrosoindole-3-acetonitrile

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Helicobacter pylori (*H. pylori*) infection and high intake of various traditional salt-preserved foods are regarded as risk factors for human gastric cancer. We previously reported that Chinese cabbage contains indole compounds, such as indole-3-acetonitrile, a mutagen precursor. 1-Nitrosoindole-3-acetonitrile (NIAN), formed by the treatment of indole-3-acetonitrile with nitrite under acidic conditions, shows direct-acting mutagenicity. In the present study, NIAN administration by gavage to Mongolian gerbils (MGs) at the dose of 100 mg/kg two times a week resulted in three adduct spots (1.6 adducts/10^8 nucleotides in total), detected in DNA samples from the glandular stomach by ^{32}P-postlabeling methods. Treatment with six consecutive doses of 100 mg/kg of NIAN, two times a week for 3 weeks, induced well—and moderately—differentiated glandular stomach adenocarcinomas in the MGs at the incidence of 31% under *H. pylori* infection at 54–104 weeks. Such lesions were not induced in MGs given broth alone, broth + NIAN or infection with *H. pylori* alone. Thus, endogenous carcinogens formed from nitrosation of indole compounds could be critical risk factors for human gastric cancer development under the influence of *H. pylori* infection.

Carcinogenesis

Gastric cancer is the second most frequent cause of cancer death worldwide.[1] Although gastric cancer has become a relatively rare cancer in North America and most Northern and Western European countries, it remains common in East Asia, Eastern Europe, Russia, and selected areas of Central and South America.[2] *Helicobacter pylori* (*H. pylori*) is a well-established major risk factor for gastric cancer,[3–5] and the prevalence of *H. pylori* infection in East Asia countries, including Japan and Korea is reported to be relatively high.[6,7] In addition, the risk of gastric cancer is increased with a high intake of various traditional salt-preserved foods.[3] In fact, pickled vegetable consumption is reported to increase gastric cancer risk in Japan and Korea.[8–10] In Korea, kimchi, commonly prepared with Chinese cabbage or radish, is a traditional and popular food, which contains high levels of nitrate (median 1550 mg/kg).[11] Furthermore, Chinese cabbage is well known as a pickled vegetable commonly consumed in Japan. Moreover, ingestion of nitrate, mainly from food, is suggested to correlate with mortality from gastric cancer.[12–14] Ingested nitrate is mainly converted to nitrite by bacteria in the oral cavity after secretion into saliva.[15] Carcinogenic *N*-nitroso compounds can be formed from nitrite and secondary amines under acidic conditions. Furthermore, direct-acting *N*-nitroso compounds, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)[16] and *N*-methyl-*N*-nitrosourea (MNU),[17] are known to induce cancer in the glandular stomach of experimental animals. Thus, it is suggested that *N*-nitroso compounds that are formed in the stomach under acidic conditions could be positively associated with the risk of gastric cancer. Nitric oxide, formed by nitric oxide synthase, is also reported to contribute to production of *N*-nitroso compounds.[18]

We have previously reported that treatments of various foodstuffs with nitrite under acidic conditions produce direct-acting mutagens towards *Salmonella* tester strains.[19,20] Among those foodstuffs, Chinese cabbage is shown to contain three indole compounds, indole-3-acetonitrile, 4-methoxyindole-3-acetonitrile and 4-methoxyindole-3-aldehyde as mutagen precursors. 1-Nitrosoindole-3-acetonitrile (NIAN), an *N*-nitroso-substituted compound formed by treatment of indole-3-

Key words: gastric cancer, *Helicobacter pylori*, Mongolian gerbil 1-nitrosoindole-3-acetonitrile, indole-3-acetonitrile

Abbreviations: DMSO: dimethyl sulfoxide; H&E: hematoxylin and eosin; *H. pylori*: *Helicobacter pylori*; MG: Mongolian gerbil; MNNG: *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU: *N*-methyl-*N*-nitrosourea; NIAN: 1-nitrosoindole-3-acetonitrile.

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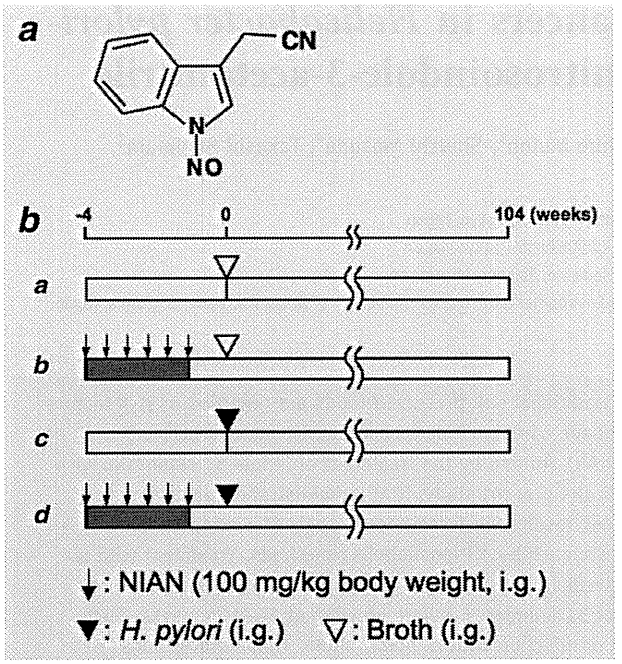

Figure 1. Chemical structure of NIAN and experimental protocol for the carcinogenicity study. (*a*) Chemical structure of NIAN. (*b*) Male 6-week-old MGs were orally administered NIAN (100 mg/kg) in 50% DMSO (groups B and D) or 50% DMSO alone (groups A and C) two times a week for 3 weeks. One week after the final administration, the animals were inoculated with *H. pylori* (ATCC 43504) (groups C and D) or sterilized broth (groups A and B).

acetonitrile with nitrite under acidic conditions, is a direct-acting mutagen in *S. typhimurium* and Chinese hamster lung cells,[20–22] and it is confirmed to form DNA adducts and to induce DNA single-strand scission in the rat glandular stomach.[23,24] Therefore, NIAN could play some role in gastric cancer development, as in the case of the well-known direct-acting mutagens, MNNG and MNU, in animal experiments.[16,17,25]

The Mongolian gerbil (MG) is reported to be susceptible to colonization by *H. pylori*, and *H. pylori* infection greatly enhances MNNG or MNU-induced gastric carcinogenesis in MGs.[26,27] Therefore, the MG is considered to be a useful animal model for evaluating the gastric cancer risk of direct-acting *N*-nitroso compounds, with or without *H. pylori* infection.

Chinese cabbage, containing nitrate and indole compounds, is commonly consumed in East Asian countries, including Japan, Korea and China, in which gastric cancer mortality is very high. In the present study, DNA adducts were detected with NIAN treatment in the glandular stomach of MGs, and the carcinogenicity of NIAN for gastric cancer *in vivo* was examined. The results clearly demonstrated that gastric cancer developed with a combination of NIAN administration and *H. pylori* infection in MGs. Possible involvement of indole compounds and nitrate derived from various foodstuffs, including Chinese cabbage, in gastric cancer development in humans is discussed.

Material and Methods

Materials

Indole-3-acetonitrile was purchased from Tokyo Food Techno (Tokyo, Japan), sodium nitrite from Wako Pure Chemical Industries (Osaka, Japan) and ammonium sulfamate from Kanto Chemical (Tokyo, Japan). Brucella broth was obtained from Becton Dickinson (Cockeysville, MD) and horse serum from Nippon Bio-Supply (Tokyo, Japan).

Preparation of NIAN

The chemical structure of NIAN is shown in Figure 1*a*. Indole-3-acetonitrile in 27 mM citrate-phosphate buffer (pH 3.0) was treated with 50 mM sodium nitrite for 1 hr at room temperature in the dark, as previously reported.[21] Nitrosation was stopped by addition of ammonium sulfamate at a final concentration of 50 mM. The reaction solution was filtered and the residue was washed with deionized water, then with *n*-hexane. The residual paste was dried and stored at −80°C until use. The preparation was >93% pure as judged by its UV absorbance on HPLC.

Bacterial culture

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was cultured in brucella broth supplemented with 10% heat-inactivated horse serum for 24 hr at 37°C under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2), as previously described.[28]

Animal treatment

Specific pathogen-free male, 6-week-old MGs (MGS/Sea, Kyudo, Fukuoka, Japan) were housed in a biohazard room, air-conditioned at 24°C ± 2°C and 55% humidity, on a 12 hr light–dark cycle and were allowed free access to commercial diet (CE-2; CLEA Japan, Tokyo, Japan) and water.

To analyze the formation of DNA adducts in the glandular stomach of MGs by NIAN treatment, NIAN was dissolved in 50% dimethyl sulfoxide (DMSO), and administered to three MGs by gavage of 0.5 ml solution, two times a week at a level of 100 mg/kg body weight. Two further MGs served as a control group receiving the solvent alone (0.5 ml). At 8 hr after administration of NIAN, both groups of animals were sacrificed under ether anesthesia, and their stomachs were resected and stored at −80°C until use. DNA was extracted by a standard procedure with enzymatic digestion of protein and RNA followed by extraction with phenol and chloroform/isoamyl alcohol (24:1, v/v).

The protocol for long-term gastric carcinogenicity in MGs treated with NIAN + *H. pylori* infection is illustrated in Figure 1*b*. The animals were randomly divided into four groups (groups A–D). Groups A and C were given 50% DMSO without NIAN (0.5 ml) whereas groups B and D were orally administrated NIAN (0.5 ml, 100 mg/kg body weight) dissolved in 50% DMSO by gavage, two times a week for 3 weeks. At one week after the last administration, the

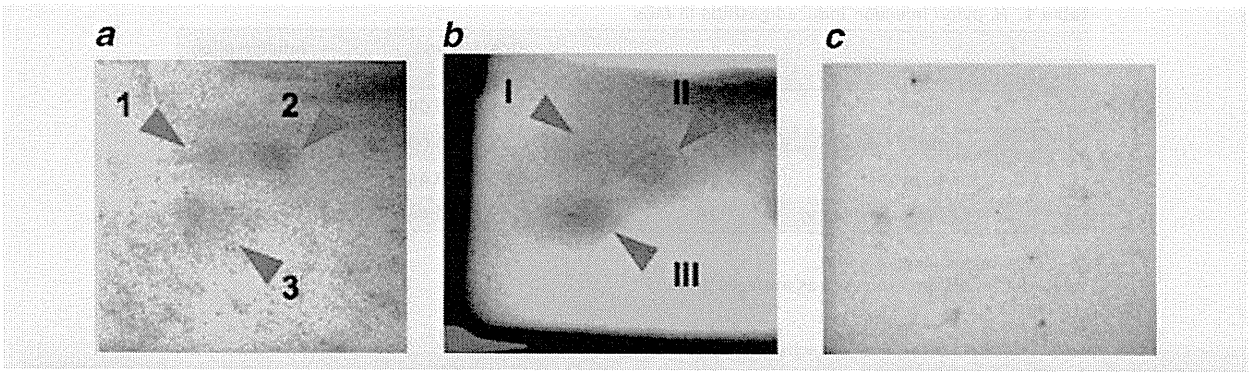

Figure 2. Autoradiograms of NIAN-DNA adducts in glandular stomach of MGs or calf thymus DNA treated with NIAN. Adducts were analyzed by ^{32}P-postlabeling method, as described in the Material and Methods. DNA samples were isolated from glandular stomach of MGs (*a*) or calf thymus DNA (*b*) after treatment with NIAN. DNA samples were also prepared from glandular stomach of MGs without NIAN treatment (*c*). Arrowheads indicate adducts. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

animals of groups C and D were given an intragastric inoculation of *H. pylori* broth culture (0.5 ml, 0.9 × 10^8 CFU/animal) whereas animals of groups A and B were given sterilized broth alone (0.5 ml).[28]

During the experiments, animals which became moribund or emaciated (<80 g body weight) were sacrificed. At 104 weeks after *H. pylori* infection, all surviving animals were sacrificed under ether anesthesia. At performance of necropsy, all tissues were carefully checked macroscopically and the stomachs and major organs were removed and assessed for macroscopic lesion development. Effective numbers of animals were defined as those surviving until week 54 of the study, when gastric tumors were observed for the first time. In addition, in the *H. pylori*-infected groups, the animals developing gastritis observed on histological examination were regarded as effective. The percentages of gastritis-bearing animals by the single inoculation of *H. pylori* were 62% for group C and 76% for group D, being similar to those previously reported.[27] All animal experiments were performed according to the "Guidelines for Animal Experiments in the National Cancer Center" and were approved by the Institutional Ethics Review Committee for Animal Experimentation in the National Cancer Center.

Detection of DNA adducts by ^{32}P-postlabeling method

Calf thymus DNA (0.5 mg, Sigma, St. Louis, MO) treated with NIAN (3 mg) for 12 hr under neutral conditions was used for authentic NIAN-DNA adducts.[23] DNA samples from the glandular stomach of MGs and calf thymus DNA samples were digested with micrococcal nuclease and phosphodiesterase II, and subjected to ^{32}P-postlabeling analysis using the same procedure as described previously[23] except with solvent systems for two-dimensional development. The solvent system consisted of buffer A (4.0 M lithium formate, 7.7 M urea, pH 3.5) from bottom to top, and buffer B (0.90 M lithium chloride, 0.45 M Tris-HCl, 7.7 M urea, pH 8.0) from left to right, followed by 1.7 M sodium phosphate buffer, pH 6.0, from left to right, with 3.5 cm filter paper. Adducts were detected with a Bio-Image Analyzer (BAS 3000; Fuji Photo Film, Tokyo, Japan) after exposing the TLC sheets to Fuji imaging plates. Relative adduct labeling was determined by the methods of Reddy *et al.*,[29] and values were calculated as averages using data from three assays.

Histological examination

All excised stomachs were opened along the greater curvature and washed twice with saline, then fixed in 10% neutral-buffered formalin. The fixed stomachs were sliced along the longitudinal axis into 9–12 strips of equal width, and routinely processed to sections stained with hematoxylin and eosin (H&E). The degree of chronic active gastritis was graded according to criteria modified from the Updated Sydney System,[30] by scoring the infiltration of neutrophils and mononuclear cells. Other organs, in which macroscopic lesions were observed, were also fixed in 10% neutral-buffered formalin and routinely processed to sections stained with H&E for histological examination.

Statistical analysis

The significance of differences in quantitative data for gastric inflammation, gastric adenocarcinoma and tumors of other organs was analyzed by Fisher's exact test. Data for stomach wet weight and inflammation score were examined using Tukey's multiple comparison test. Significance was concluded at $p < 0.05$.

Results

DNA adduct formation by NIAN administration in the glandular stomach of MGs

To confirm the formation of NIAN-DNA adducts in the glandular stomach of MGs, NIAN was injected two times a week at a dose of 100 mg/kg by gavage, and then analyzed by ^{32}P-postlabeling method. Three adduct spots were observed in DNA samples derived from NIAN-treated animals (Fig. 2*a*). The adduct levels were 0.3 for adduct 1, 1.1 for adduct 2, 0.2 for adduct 3 and 1.6 adducts/10^8 nucleotides

Table 1. *H. pylori* infection induced-gastritis in MGs

Group	Treatment	Effective No.	Stomach wet weight (g)	Inflammation score
A	Broth	15	0.647 ± 0.097	0
B	NIAN + Broth	22	0.631 ± 0.094	0
C	*H. pylori*	18	1.432 ± 0.445*	2.22 ± 0.43*
D	NIAN + *H. pylori*	26	1.483 ± 0.445*	2.38 ± 0.64*

*$p < 0.01$ versus group A and B.
Values for results are expressed as averages ± SD.

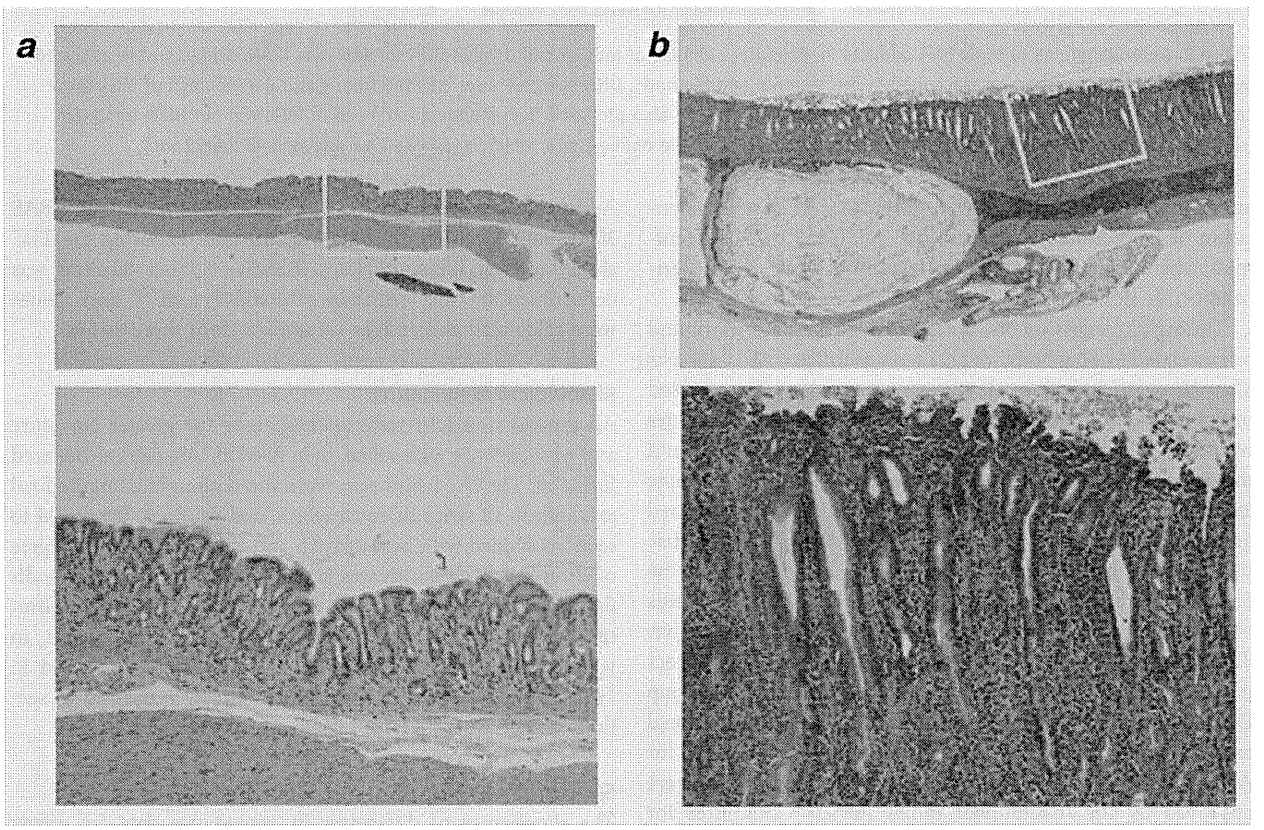

Figure 3. Macroscopic and microscopic views of gastritis in MGs infected or uninfected with *H. pylori*. (*a*) Normal gastric mucosa in group A. (*b*) Severe infiltration of many inflammatory cells with development of heterophilic proliferative glands in group C; H&E staining, ×40. Yellow boxes are shown at greater magnification below, ×200.

in total. This TLC pattern was similar to that in the *in vitro* reaction of calf thymus DNA with NIAN (total adduct level of 4.8 adducts/10^7 nucleotides, Fig. 2*b*). In the case of DNA samples derived from control animals, no adduct spots were seen on the TLC sheets (Fig. 2*c*).

Macroscopical and microscopical observation of *H. pylori*-induced gastritis in MGs

MGs were sacrificed until 104 weeks after *H. pylori* infection, and gastric disorders were analyzed. Stomach wet weights and gastric inflammation scores are shown in Table 1. Macroscopically, edematous thickening with hemorrhagic spots was apparent in the gastric mucosa in *H. pylori*-infected MGs (groups C and D), but not in animals uninfected with *H. pylori* (groups A and B). The stomach wet weight, reflecting edematous thickening, in animals infected with *H. pylori* (groups C and D) was significantly increased compared with that of animals not infected with *H. pylori* (groups A and B) ($p < 0.01$). No significant differences of stomach wet weight were detected between groups A and B and also between groups C and D.

Microscopically, gastritis, featuring infiltration of many inflammatory cells, and hyperplastic change of glandular epithelium, and erosion were observed in the pyloric regions of

Table 2. Incidence of glandular stomach adenocarcinoma in MGs

Group	Treatment	Effective No.	No. of animals with glandular stomach adenocarcinoma (%)		
			Total	Well dif.	Moderately dif.
A	Broth	15	0 (0)	0 (0)	0 (0)
B	NIAN + Broth	22	0 (0)	0 (0)	0 (0)
C	*H. pylori*	18	0 (0)	0 (0)	0 (0)
D	NIAN + *H. pylori*	26	8 (31)*	7 (27)	1 (4)

Well dif., well differentiated adenocarcinoma; Moderately dif., moderately differentiated adenocarcinoma.
*$p < 0.05$ versus group A and C and $p < 0.01$ versus group B.

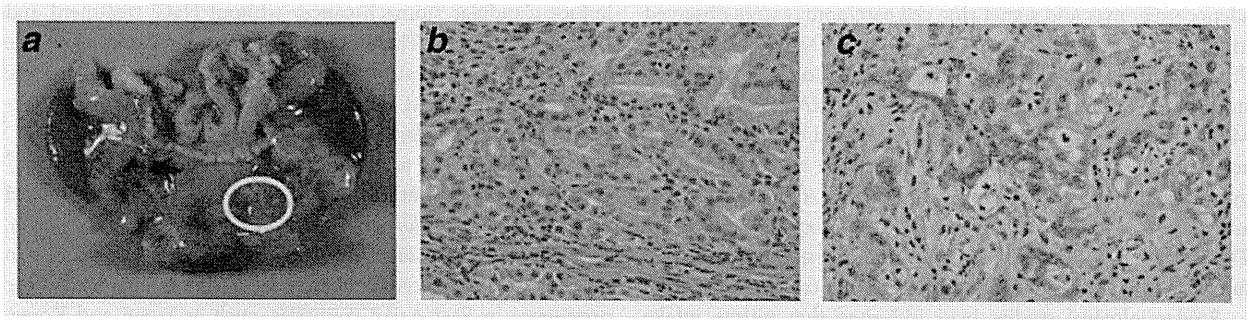

Figure 4. Histological findings of gastric adenocarcinoma in the animals treated with both NIAN and *H. pylori*. (*a*) Typical macrograph of a stomach. The yellow circle shows the suspected lesion of gastric cancer. (*b*) Well differentiated adenocarcinoma. (*c*) Moderately differentiated adenocarcinoma. (*b* and *c*) H&E staining, ×400.

the animals infected with *H. pylori* (groups C and D) (Fig. 3). Heterotopic proliferative glands, whose development is related to severe gastritis in *H. pylori*-infected MGs, were sometimes observed in *H. pylori*-infected groups (groups C and D). No gastritis was found in animals not infected with *H. pylori* (groups A and B). The gastric inflammation score in *H. pylori*-infected animals was significantly increased compared with that of animals uninfected with *H. pylori* ($p < 0.01$). There were no significant differences of gastric inflammation score between groups C and D.

Development of glandular stomach adenocarcinomas in MGs treated with both NIAN and *H. pylori*

The observed incidences of glandular stomach adenocarcinomas are shown in Table 2. Glandular stomach adenocarcinomas, histologically featuring tubular structures with cellular atypia infiltrating into the muscle layer, were found in eight animals treated with both NIAN and *H. pylori* (8/26 = 31%) at 54–104 weeks. All adenocarcinomas were observed in the pyloric mucosa and located in the lesser curvature of the stomach, where macroscopically severe edematous thickening was also seen (Fig. 4*a*). The observed adenocarcinomas in seven animals were of well differentiated (Fig. 4*b*), and a moderately differentiated lesion was observed in one animal (Fig. 4*c*). In the animals treated with broth alone, broth + NIAN and *H. pylori* alone (groups A, B and C), no glandular stomach adenocarcinomas were observed. The incidence of glandular stomach adenocarcinomas in group D was significantly higher than that in groups A, B and C ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively).

Irrespective of NIAN treatment and *H. pylori* infection, skin tumors, which histologically were well to poor differentiated squamous cell carcinomas, sebaceous carcinomas and melanomas, were found in one animal (1/15 = 7%) in group A, three animals (3/22 = 14%) in group B, two animals (2/18 = 11%) in group C and five animals (5/26 = 19%) in group D. A hemangioma was also observed in a kidney of one animal in group D (1/26 = 4%). No significant differences were apparent in these tumor incidences among groups A–D.

Discussion

In the present study, NIAN was found to induce glandular stomach adenocarcinomas in MGs in combination with *H. pylori* infection. NIAN-DNA adducts were also detected in the glandular stomach of MGs after treatment with NIAN, although clarification of their chemical structure(s) has yet to be performed. DNA adducts observed in the glandular stomachs of NIAN-treated MGs probably contain an indole-3-acetonitrile moiety. However, it is further likely that NIAN would act as an NO donor under aqueous conditions, thereby causing DNA modifications.[31–33] In fact, Lucas *et al.* demonstrated that NIAN can efficiently transfer nitroso groups to nucleophilic targets in purine nucleotides, causing *N*-nitrosation, deamination and the formation of a novel guanine analog, oxanine.[33]