

# Cross-sectional Study with Multiple Measurements of Biological Markers for Assessing Stomach Cancer Risks at the Population Level

by Shchoichiro Tsugane,<sup>1</sup> Mitsuhiro Tsuda,<sup>2</sup> Fred Gey,<sup>3</sup> and Shaw Watanabe<sup>1</sup>

A cross-sectional study to determine correlations between measurable biologic markers and mortality from stomach cancer was performed in various areas of Japan. Blood and urine were collected from randomly selected 40- to 49-year-old men and their spouses in four areas with different rates of mortality from stomach cancer. The samples were analyzed for levels of the micronutrients vitamins A, C, and E,  $\beta$ -carotene, and lycopene in plasma and for levels of NaCl, nitrate, and *N*-nitrosamino acids (*N*-nitrosoproline, *N*-nitrosothioproline [NTPRO] and *N*-nitrosomethylthioproline [NMTPRO]) in 24-hr urine. A significant, strong correlation was found between the amount of salt excreted in urine and stomach cancer mortality in both men and women. Although the amounts of nitrate and of the three *N*-nitrosamino acids in 24-hr urine were not correlated with stomach cancer rates, the low excretion levels of NTPRO and NMTPRO in the lowest risk area for stomach cancer were noteworthy, regardless of the high level of nitrate excretion in the same area. This suggests a lesser degree of endogenous nitrosation in the body. No protective effect of micronutrients was observed in this correlation study; there was, however, a negative correlation between plasma lycopene level and stomach cancer mortality. Salt intake was thus confirmed to play an important role in the development of stomach cancer and is likely to be a rate-regulating factor in Japanese populations. *N*-Nitrosamino acids and lycopene may also be related to stomach cancer mortality.

## Introduction

The geographic distribution of mortality from stomach cancer is quite different even among the homogeneous population of Japan. Such geographic differences suggest that the risk factors for this cancer are related to lifestyle factors, and especially to dietary habits. The purpose of this study was to clarify the importance of potential risk factors for stomach cancer in representative populations in four areas of Japan by measuring several selected biologic markers and correlating them with the rate of stomach cancer mortality. If rate-regulating risk factors for stomach cancer could be identified, appropriate preventive measures could be applied.

## Study Area

Each study area has a population of approximately 100,000 people and is served by a single prefectural health center, which supervises the health administration of several cities, towns, and villages in the area. Four areas were selected for this study on the basis of age-adjusted mortality rates for stomach cancer: Yokote in Akita Prefecture has the highest rate; Saku in Nagano Prefecture has the next highest rate; Ninohe in Iwate Prefecture has a lower rate; and Ishikawa in Okinawa Prefecture has the lowest rate. Geographic locations and age-adjusted mortality rates from 1985 to 1987 for stomach cancer (adjusted to the 100,000 world population) in these areas are shown in Figure 1.

## Subjects

In each area, 170-175 men, aged 40-49 years, were selected by random sampling from resident registration lists. The purpose of the study was described, and subjects were asked for voluntary participation. Spouses of participating men were also invited to participate in the study. The rates of participation were 78% (133/170) in Yokote,

<sup>1</sup>Epidemiology Division, National Cancer Center Research Institute 5-1-1, Tsukiji, Chuo-ku, Tokyo 104, Japan.

<sup>2</sup>Biochemistry Division, National Cancer Center Research Institute 5-1-1, Tsukiji, Chuo-ku, Tokyo 104, Japan.

<sup>3</sup>Vitamin Unit, Institute of Biochemistry and Molecular Biology, University of Bern, Switzerland.

Address reprint requests to S. Tsugane, National Cancer Center Research Institute 5-1-1, Tsukiji, Chuo-ku, Tokyo 104, Japan.

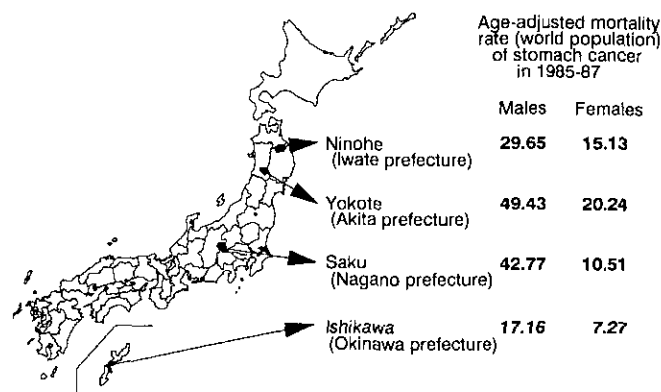


FIGURE 1. Location of survey areas and age-adjusted mortality rates for stomach cancer (adjusted to standard world population, per 100,000).

71% (120/170) in Saku, 77% (134/175) in Ninohe, and 76% (129/170) in Ishikawa; 52-72% of spouses participated in the study. The population of each area and the numbers of participants are shown in Table 1.

Blood and 24-hr urine samples were taken between February and March 1989 in the Ninohe and Ishikawa areas and in 1990 in the Yokote and Saku areas.

## Collection of Biologic Specimens

Blood samples were taken from all participants after they had fasted for at least 5 hr. Plasma was separated immediately from whole blood by centrifuge and stored in several microtubes with dry ice or in a deep-freeze at  $-80^{\circ}\text{C}$  until time for chemical analyses; the time between sampling and freezing was  $< 30$  min. *Meta*-phosphoric acid was added to one microtube to stabilize vitamin C.

Twenty-four-hour urine samples were collected voluntarily from participants on any day except Saturdays,

Sundays, or holidays. A total of 156 men and 134 women provided urine samples (Table 1). Samples in which the daily creatinine excretion was less than 0.5 g for men and less than 0.4 g for women were assumed to represent incomplete urine collection and were not included in the analysis.

## Biologic Markers in Urine and Plasma Salt in 24-hr Urine

Several experimental and epidemiologic findings support an etiologic role for salt in the development of stomach cancer. Various indicators of salt intake have been used in epidemiologic studies. In most case-control and cohort studies, intake frequency of highly salted foods and salt preference have been used, but measurement of excreted salt in 24-hr urine is considered to be one of the best markers for evaluating daily salt intake.

Median and mean values, with standard deviations, of salt in 24-hr urine samples are shown in Table 2. These values correlated well with the mortality rate of stomach cancer in each area (Fig. 2). This strong correlation suggests that salt consumption has important and potentially rate-regulating effects for the development of stomach cancer in the Japanese population.

## Nitrate and *N*-Nitrosamino Acids in 24-hr Urine

*N*-Nitroso compounds, specifically *N*-nitrosamides, are powerful carcinogens and induce stomach cancer in animal models. Nitrosamides are synthesized in the human stomach from nitrite and alkyl amides, both of which are derived from common foods. Levels of exposure to these substances must be estimated for evaluating stomach cancer risk. We analyzed the urinary nitrosamino acids

Table 1. Populations in each study area and numbers of participants.

Area	Population in 1987	No. of males age 40-49	No. of subjects selected	No. of subjects participating (%)	No. from whom 24-hr urine samples were collected	No. of spouses participating	No. from whom 24-hr urine samples were collected
Yokote	77,755	4,583	170	133 (78)	43	80	30
Saku	118,002	7,042	170	120 (71)	48	79	39
Ninohe	108,668	6,619	175	134 (77)	33	70	33
Ishikawa	125,711	7,287	170	129 (76)	32	93	32

Table 2. Median and mean ( $\pm$  SD) for various chemical markers in 24-hr urine.

Area	No. of samples	NaCl, g		Nitrate, mg		NPRO, $\mu\text{g}$		NTPRO, $\mu\text{g}$		NMTPRO, $\mu\text{g}$	
		Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean
Males											
Yokote	37	12.2	13.4 $\pm$ 6.6	155	166 $\pm$ 100	1.9	4.4 $\pm$ 5.5	11.5	19.1 $\pm$ 21.1	7.4	10.0 $\pm$ 11.7
Saku	38	11.4	11.9 $\pm$ 5.3	98	120 $\pm$ 64	2.8	3.7 $\pm$ 3.6	9.3	12.6 $\pm$ 16.2	6.8	11.8 $\pm$ 10.4
Ninohe	28	9.2	9.9 $\pm$ 3.6	148	167 $\pm$ 99	3.1	3.5 $\pm$ 2.3	13.4	17.3 $\pm$ 15.6	8.6	12.3 $\pm$ 13.8
Ishikawa	29	7.1	8.0 $\pm$ 3.2	171	203 $\pm$ 111	3.2	4.9 $\pm$ 4.3	7.7	10.3 $\pm$ 8.7	3.6	5.0 $\pm$ 5.6
Females											
Yokote	24	11.4	11.9 $\pm$ 4.8	162	187 $\pm$ 113	2.7	5.9 $\pm$ 14.3	9.2	17.1 $\pm$ 19.2	5.2	7.3 $\pm$ 5.0
Saku	33	8.4	9.1 $\pm$ 3.7	124	136 $\pm$ 80	1.9	2.6 $\pm$ 1.8	9.2	11.3 $\pm$ 8.3	6.6	9.9 $\pm$ 9.3
Ninohe	26	9.9	10.1 $\pm$ 3.2	231	218 $\pm$ 82	3.8	4.3 $\pm$ 3.1	13.8	18.9 $\pm$ 15.5	5.4	7.5 $\pm$ 6.1
Ishikawa	28	7.7	7.7 $\pm$ 2.7	153	151 $\pm$ 98	2.1	3.3 $\pm$ 3.1	5.5	6.6 $\pm$ 5.4	1.0	1.6 $\pm$ 2.0

Abbreviations: NPRO, *N*-nitrosoproline; NTPRO, *N*-nitrosothioproline; NMTPRO, *N*-nitrosomethylthioproline.

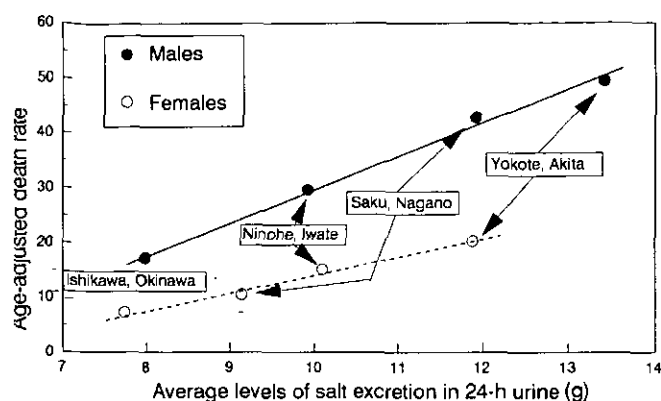


FIGURE 2. Correlation between salt excretion in 24-hr urine and stomach cancer mortality.

*N*-nitrosoproline, *N*-nitrosothiopropine (NTPRO), and *N*-nitrosomethylthiopropine (NMTPRO) as indicators of endogenous nitrosation by gas chromatography–thermal energy analysis (1). The values for nitrate and for the three *N*-nitrosamino acids (Table 2) did not correlate well with stomach cancer mortality. Even though nitrate excretion was found to be high in both men and women in Ishikawa, values for NTPRO and NMTPRO were strikingly low in this lowest risk area for stomach cancer.

Since most *N*-nitrosamino acids are considered to be formed endogeneously by reaction with nitrite from

nitrate, the correlation coefficients between nitrate and the three nitrosamines are shown, in Table 3. Positive, statistically significant correlations were observed in more than half of the associations examined. When the correlations between nitrate and NTPRO were plotted for the area with the highest rate of mortality from stomach cancer (Yokote) and for the lowest (Ishikawa) and regression lines were drawn (Fig. 3), both areas showed a significant correlation, with coefficients of about 0.5; however, the slope was steeper for Yokote than for Ishikawa for both men and women. These findings led us to suggest that the extremely low mortality from stomach cancer in Ishikawa could be related to a lesser degree of endogenous formation of carcinogenic *N*-nitrosamines. This would be related to a smaller amount of precursor amines in foods or decreased reactivity between nitrite and amines owing to gastric pH (2).

## Antioxidant Vitamins and Carotenoids in Plasma

Many epidemiologic studies of stomach cancer have shown a protective role of green–yellow vegetables and fruits. These effects are considered to be due to the antioxidant action of micronutrients such as  $\beta$ -carotene and vitamin C or to an inhibitory action of vitamin C on endogenous nitrosation. Antioxidants were measured only for men in this study, as a part of an “Optional Study on

Table 3. Spearman rank correlation coefficients of three *N*-nitrosamino acids with nitrate.

Area	NPRO		NTPRO		NMTPRO	
	Males	Females	Males	Females	Males	Females
Yokote	0.09	0.32	0.50†	0.46*	0.46†	0.02
Saku	0.08	0.45†	0.24	0.27	0.35*	0.35*
Ninohe	0.29	0.30	0.35	0.39	0.21	0.50*
Ishikawa	0.41*	0.53†	0.59‡	0.43†	0.50†	0.41*

Abbreviations: NPRO, *N*-nitrosoproline; NTPRO, *N*-nitrosothiopropine; NMTPRO, *N*-nitrosomethylthiopropine.

\* $p < 0.05$ .

† $p < 0.01$ .

‡ $p < 0.001$ .

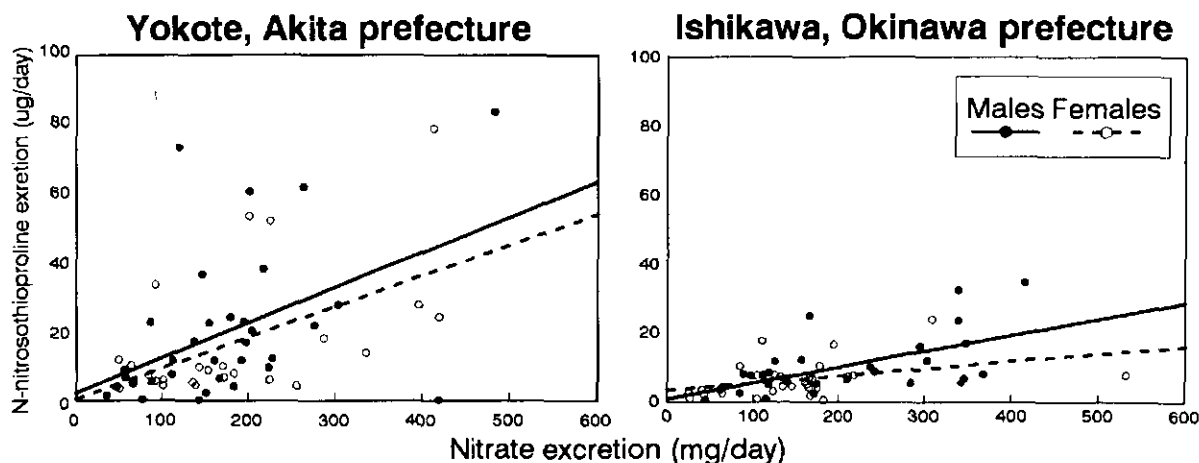


FIGURE 3. Correlations between excretion of nitrate and of *N*-nitrosothiopropine in 24-hr urine in areas of high (Yokote) and low (Ishikawa) mortality from stomach cancer.

**Table 4. Median and mean ( $\pm$  SD) levels of plasma micronutrients ( $\mu$ mole/L) in men.**

Area	No. of samples	Vitamin A		Vitamin C		Vitamin E		$\beta$ -Carotene		Lycopene	
		Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean
Yokote	132	2.8	2.8 $\pm$ 0.6	45	45 $\pm$ 14	22	23 $\pm$ 7	0.14	0.20 $\pm$ 0.17	0.06	0.08 $\pm$ 0.07
Saku	114	2.5	2.5 $\pm$ 0.6	48	47 $\pm$ 13	23	24 $\pm$ 7	0.28	0.36 $\pm$ 0.31	0.13	0.15 $\pm$ 0.11
Ninohe	131	2.3	2.4 $\pm$ 0.5	45	46 $\pm$ 15	22	23 $\pm$ 7	0.24	0.31 $\pm$ 0.24	0.09	0.12 $\pm$ 0.13
Ishikawa	128	2.2	2.2 $\pm$ 0.6	36	35 $\pm$ 15	25	27 $\pm$ 9	0.23	0.29 $\pm$ 0.22	0.22	0.27 $\pm$ 0.21

Antioxidant Vitamins and PUFAs, WHO/MONICA Project," which was organized by one of authors (F. G.) (3).

Plasma concentration of vitamins A, E, and C and of carotenoids such as  $\beta$ -carotene and lycopene are shown in Table 4. The levels of  $\beta$ -carotene and lycopene were lowest in the highest risk area (Yokote), and that of lycopene was highest in the lowest risk area (Ishikawa). Lycopene has the strongest quenching potency for singlet oxygen among various carotenoids and xanthophylls, and its overall quenching capacity is three times higher than that of  $\beta$ -carotene and  $\alpha$ -tocopherol in human plasma (4). This inverse correlation may indicate a protective effect of lycopene against stomach cancer in this Japanese population.

The plasma vitamin C level was not correlated with stomach cancer mortality, and it was the lowest in the lowest risk area (Ishikawa). The low level of nitrosation in this area therefore cannot be related to an inhibitory role of vitamin C, although the plasma level of this micronutrient may not always reflect its level in gastric juice (5).

## Conclusion

Urinary salt excretion was the best predictor for the different rates of stomach cancer mortality in the four regional subpopulations of Japan studied here. Although salt itself is not carcinogenic, salt consumption may modify the probability that an initiated cell will progress to malignancy. Endogenous formation of *N*-nitrosamines and the antioxidant action of micronutrients, such as lycopene, may also play important roles in the regulation of stomach cancer incidence.

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