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DIETARY EXPOSURE TO NITRITE AND NITROSAMINES AND RISK OF NASOPHARYNGEAL CARCINOMA IN TAIWAN

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Previous studies of nasopharyngeal carcinoma (NPC) have found elevated risks with higher consumption of salted fish and preserved foods, particularly during childhood. These foods can contain high levels of nitrosamines; however, most studies have not estimated exposure to nitrosamines directly. We conducted a case-control study in Taiwan to evaluate dietary intakes and NPC risk. A total of 375 cases (99% response rate) and 327 controls (88% response rate) were interviewed about their diet as an adult and at age 10 using a food-frequency questionnaire. We interviewed mothers of participants about their child's diet at age 10, age 3 and during weaning and the mother's diet while she was breast-feeding. Mothers of 96 cases and 120 controls were interviewed. Nitrosamine and nitrite levels were assigned to 66 foods based on published values. Intake of nitrosamines and nitrite as an adult was not associated with risk of NPC. High intakes of nitrosamines and nitrite during childhood and weaning were associated with increased risks of NPC for foods other than soy products. Adjusted odds ratios for the highest quartile were 2.2 [95% confidence interval (CI) 0.8–5.6] for age 10, 2.6 (95% CI 1.0–7.0) for age 3 and 3.9 (95% CI 1.4–10.4) for weaning diet. Intakes of nitrite and nitrosamines from soybean products during childhood and weaning were inversely associated with risk. Soybeans contain known inhibitors of nitrosation, and thus may explain the inverse association we observed. Our results suggest that nitrosamine and nitrite intake during childhood may play a role in the development of NPC. *Int. J. Cancer* 86:603–609, 2000.
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Nasopharyngeal carcinoma (NPC) is rare in most parts of the world, but incidence is high in China and southeast Asia among individuals of Chinese descent. Infection with Epstein-Barr virus (EBV), smoking, some occupational exposures and diet have been linked to risk (Hildesheim and Levine, 1993). Host factors suspected to be related to risk include specific human leukocyte antigens and the cytochrome P4502E1 (CYP2E1) genotype. Most studies that have evaluated dietary intakes have focused on local foods eaten in high-risk areas, including salted fish, various preserved foods and hot spices. Consumption of these foods, particularly during weaning or at a young age, appears to be an important risk factor (World Cancer Research Fund, 1997; Yu *et al.*, 1988, 1989*a*; Ning *et al.*, 1990).

Salted fish and other salted and preserved foods contain nitrosamines and nitrosamine precursors, known animal carcinogens (Fong and Chan, 1977; Poirier *et al.*, 1987). Chinese salted fish can cause nasal cavity tumors in rats (Yu *et al.*, 1989*b*; Huang *et al.*, 1978). Previous studies of NPC and diet have evaluated salted fish or other individual preserved foods but have not estimated the intake of nitrosamines and nitrosamine precursors summed over all dietary sources. Several studies of stomach cancer and upper aerodigestive cancer have evaluated exposure to nitrosamines and nitrosamine precursors by assigning reported levels to the food-frequency questionnaires (Rogers *et al.*, 1993; Risch *et al.*, 1985). The advantage of an assessment over all foods is that all dietary sources of the compound of interest are included. This is especially important when exposure can come from many food sources.

We estimated intake of nitrosamines, nitrite and nitrate using a comprehensive food-frequency questionnaire in a study of NPC in Taiwan. We estimated the risk of NPC with dietary exposures as an adult, at several ages during childhood and for the mother's diet during breast-feeding. We also evaluated the possible interaction of nitrite and nitrosamine intake with dietary inhibitors of nitrosamine formation, including intake of fruits, vegetables and soybean products.

MATERIAL AND METHODS

Study population

The method for case ascertainment and control selection was described in detail previously (Hildesheim *et al.*, 1997). Briefly, incident cases of NPC less than 75 years of age were identified from July 15, 1991, through December 31, 1994, from 2 referral hospitals in Taipei, Taiwan. Three hundred and seventy-eight histologically confirmed cases were identified. One population control per case was selected from the same district or township as the cases using the National Household Registration System and matched to the cases by gender and age in 5-year groups. Three hundred and seventy-four controls were identified.

Personal interviews collected information about socio-demographic characteristics, cigarette smoking, alcohol consumption, adult and childhood diet, occupation and family history of cancer. A total of 375 cases (99.2%) and 327 controls (88%) consented to an interview. The study was approved by the Institutional Review Boards at the National Cancer Institute and the National Taiwan University.

We interviewed mothers of cases and controls about children's diets at age 10, age 3 and during weaning and about the mother's diet while breast-feeding. Two hundred and three mothers of cases and 190 mothers of controls were alive at the time of the study. Among these eligible mothers, 96 (47.3%) case mothers and 120 (63.2%) control mothers completed an interview. The major reason for non-response was refusal of cases and controls to provide contact information for their mothers (cases 42.9%, controls 26.8%). Other reasons for non-response included the mother's residence outside of Taiwan (cases 3.0%, controls 3.7%) and refusal of participation by mothers (cases 7%, controls 6.3%).

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Food-frequency questionnaire

The food-frequency questionnaire included questions about the consumption of 66 foods as an adult (for the time period 10 to 3 years before the interview) and 33 foods at age 10. Mothers were interviewed about children's consumption of 33 foods at age 10, 31 foods at age 3 and 25 foods during weaning. Mothers were also asked about 34 foods they consumed while breast-feeding. The reduced number of foods during childhood was due both to the grouping of similar foods such as fresh vegetables and fruits and to the elimination of foods that were unlikely to have been consumed. Frequency of intake was ascertained in 5 possible categories: daily, weekly, monthly, yearly or less than once per year. To calculate intake for food groups, the frequencies were converted to times per week as follows: 7/week for daily, 1/week for weekly, 0.25/week for monthly, 0.02/week for yearly and 0/week for less than once per year.

Foods that were likely to contain high levels of nitrosamines and nitrite were grouped by type (meats, fish, soybean products, preserved vegetables). The food groups included cured or salted meats and duck; smoked meats, chicken and fish; salted fish; processed fish and seafood, including sauces; fermented soybean products and fermented rice; and fermented and pickled vegetables. Intakes of some of the cured meats may have been counted twice because some of the cured meats were also smoked. We evaluated intake of both moldy and firm types of Guandong salted fish for adults and children aged 10, as reported by the subject. The numbers of subjects with childhood intake as reported by the mother were too few to evaluate intake of each type separately, so we combined both types. Intake of additional foods that were potential sources of nitrosamines or salt was also evaluated, including hot chili sauce, preserved eggs and salted eggs. Due to the shorter list of foods for the childhood questionnaire, the food groups differed somewhat by the age at which diet was assessed (Appendix).

Adult intake of vegetables and fruits was assessed by asking about 10 fresh vegetables, 6 fruits and fruit juices, as well as by asking 2 general questions about the usual frequency of intake of fruits and fresh vegetables overall. For childhood intake, only the 2 general questions were asked. Adult and childhood intakes of fresh soybean products, which included tofu, other soybean curd products, soy milk and soybeans, were also evaluated.

Nitrosamines, nitrite and nitrate in foods

Numerous studies have measured levels of volatile nitrosamines in preserved and fermented foods (Zou *et al.,* 1994; Poirier *et al.,* 1987; Gao *et al.,* 1991; Siddiqi *et al.,* 1988; Fong and Chan, 1977). One comprehensive survey of nitrosamine levels in Chinese foods (Song and Hu, 1988) included measurements of cured meats, other meats, seafood, soy sauce, fermented bean curd, pickled and salted vegetables and other miscellaneous foods. For each type of food on the questionnaire, we used the median of published values (or the mean if that was the only value reported) to categorize by the level of nitrosamines. For salted fish, we used values from an analysis of steamed salted fish from various areas of China (Zou *et al.,* 1994) because steaming was the usual preparation method in Taiwan, particularly for children (W.-H.Pan, personal communication). We categorized foods as low in nitrosamines for values of 0, medium low for values $>$ 0 to 1 ppb, medium high for values $>$ 1 to $<$ 5 ppb and high for values of $\geq$5 ppb. When more than 1 nitrosamine was measured, we used the median of the highest reported values to categorize the food. If there were no nitrosamine levels reported for a food, we assigned it to a category based on foods thought to be similar. For example, nitrosamine levels in fresh Chinese vegetables and fruits were assumed to be 0, as reported for Western fresh fruits and vegetables (National Academy of Sciences, 1981).

There are few data on nitrite and nitrate levels in Chinese foods. One study measured nitrate levels in Chinese vegetables (Lin and Yen, 1980); another measured nitrite and nitrate levels in salted fish (Zou *et al.,* 1994). Nitrite levels for Japanese foods were reported in one comprehensive survey (Harada *et al.,* 1972), but most of the published values for nitrite and nitrate come from foods in Europe or the United States (National Academy of Sciences, 1981). We used the values for Chinese foods when available and estimated levels for other foods using values from Japanese or Western foods thought to be similar. For example, root vegetables and dark green vegetables have high levels of nitrate (National Academy of Sciences, 1981). Therefore, Chinese dark green leafy vegetables were also assumed to have high nitrate levels. Nitrite was categorized as low for values of 0 to $<$1 ppm, medium for values of 1 to 9 ppm and high for values $\geq$ 10 ppm. We categorized foods into the low nitrate group for values of $<$50 ppm, medium for values of 50 to $<$500 ppm and high for values of $\geq$500 ppm. Dietary sources of nitrate are primarily from vegetables (National Academy of Sciences, 1981). Because the childhood dietary interviews did not contain detailed questions about vegetable intake, we were not able to estimate nitrate intake during childhood.

Total calories were calculated from the food-frequency questionnaire for adult dietary intakes using a database specific to the Taiwanese diet (Lee *et al.,* 1992). Total calories were not calculated for childhood diet because of limited dietary information.

Data analysis

The adult dietary analyses were limited to 371 cases and 321 controls who had $<$20% missing or unknown values for the foods consumed as an adult (excluded 6 controls and 4 cases). We multiplied the reported frequency of consumption of each food by the estimated portion size in grams and converted all intakes into grams/week. For the food group analyses, intake was summed across all foods. Foods were grouped by general type (fish, meats, soybean products, vegetables) and whether they were fermented and/or salted.

We also grouped foods by nitrosamine, nitrite and nitrate level as described above and calculated risk by frequency of intake for each level. The results were similar to those for the nitrite and nitrosamine indices (described below), so they are not presented here.

Nitrate, nitrite and nitrosamine indices were calculated by multiplying the intake of each food (g/week) by a score (or weight) that depended on the level category for the food. Thus, each index ranked individuals according to intake of nitrosamines, nitrite and nitrate; higher index values indicated higher intake. Low nitrosamine and nitrite categories were given a score of 0 because foods in those categories had very low or no measurable nitrosamines or nitrite. Scores for the high and medium high levels were approximately the ratio of the midpoint of the category compared to the midpoint of the next lower category. Nitrosamine scores were 1 for medium low, 8 for medium high and 40 for high. Scores were 1 for medium nitrite and 4 for high nitrite. The low nitrate category was assigned a score of 1, medium was 25 and high was 150. Nitrite and nitrosamine indices were calculated separately for soybean products and other foods because childhood and weaning consumption of soybean products showed an inverse association with risk in the food group analysis. Index values were categorized into quartiles based on the distribution among the controls, and those in the lowest quartile were used as the referent group.

Odds ratios (ORs) and 95 percent confidence intervals (CIs) were calculated using unconditional logistic regression analysis (Dixon *et al.,* 1990), and all analyses were adjusted for age (adults: $<$40, 40–55, $>$55; subjects with mothers questionnaire: $<$40, 40+) and ethnicity (Fujian; Hakka/Aboriginal/Guandong; other Han). The adult dietary analyses were also adjusted for total calories. Fruits, vegetables, and fresh soybeans, which were inversely associated with risk are inhibitors of endogenous formation of *N*-nitroso compounds and were evaluated as potential confounders and effect modifiers of the nitrite and nitrosamine associations. Effect modification was evaluated for intake levels above and at or below the median, based on the distribution among controls. The CYP2E1 genotype was determined, but we were not able to evaluate effect modification by genotype due to the low prevalence of homozygosity (Hildesheim *et al.*, 1997). Long-term cigarette smoking was associated with an increased risk of NPC (25 years or more compared to non-smokers, OR = 1.7, 95% CI 1.1–2.9); however, adjustment for smoking did not change the ORs for nitrite and nitrosamine intakes. Therefore, the smoking-adjusted ORs are not presented here.

RESULTS

Subgroup with mother's interview

Cases and controls in the subgroup with a mother's interview tended to be younger than the study population overall due to the fact that their mothers had to be alive. Fewer controls were female (24% *vs.* 31% overall), whereas slightly more cases were female (37% *vs.* 30% overall). Compared to the entire study population, fewer cases were of Hakka, Aboriginal or Guandong ethnicity (6% *vs.* 13% overall), whereas a greater percent of controls were members of these ethnic groups (18% *vs.* 11% overall). We compared results for self-reported adult and age 10 dietary intakes among those with and without a mother's interview and found no substantial differences in the adjusted ORs for the food groups or the nitrite and nitrosamine indices.

Adult dietary intakes

Consumption of moldy and firm types of Guandong salted fish was uncommon among adults (<5% of controls), and neither type was associated with risk of NPC. Other types of salted fish were eaten more commonly but also showed no association with risk (5 g/week, OR = 0.8, 95% CI 0.5–1.2). Consumption of cured meats, smoked meats, fermented soybean products and preserved vegetables was not associated with risk of NPC. The highest quartile of intake of salted eggs was associated with a significantly elevated risk of NPC compared to the lowest intake quartile (OR = 2.0, 95% CI 1.2–3.2). The highest quartile of intake of hot chili sauce was associated with a 40% increased risk (95% CI 0.9–2.1).

Intake of fresh soybean products was not associated with risk. Intakes of all vegetables and dark green vegetables were inversely associated with risk. ORs for the third and fourth quartiles of total vegetable intake were 0.6 (95% CI 0.4–1.0) and 0.6 (95% CI 0.4–1.0), respectively. Dark green vegetables accounted for about half of the vegetable intake, and risks were similar to total vegetable intake. Fruit intake was not associated with risk.

We found no association between the quartile levels of the nitrosamine and nitrite indices (hereafter referred to as intakes) and risk when the indices were calculated from all foods. Nitrite and nitrosamine intake from soy products, which constituted a small proportion of total intake during adulthood, were not associated with risk of NPC. Higher intakes of nitrite and nitrosamines from other foods, which included preserved meats, fish and vegetables, were associated with slightly decreased risks, though none of the ORs was significant. There was no evidence of effect modification by intake of fruits, vegetables or fresh soybeans.

High nitrate intake as an adult was associated with a significantly decreased risk of NPC (OR = 0.5, 95% 0.3–0.8). Consumption came primarily from fresh and preserved vegetables.

Dietary intake during childhood and weaning

Food groups. We compared the results for food group intakes at age 10 as reported by the mother with those reported by subjects. The subject-reported intake was generally higher than that reported by mothers. For every food or food group, with the exception of hot chili sauce and smoked meats, the association between quartiles of intake and risk was consistent between mothers' and subjects' reports. High intakes of hot chili sauce and smoked meats as reported by the subjects were associated with increased risks of NPC (chili sauce OR = 2.3, 95% CI 1.1–4.7; smoked meats OR = 1.8, 95% CI 0.9–3.5), whereas there was no association with intake as reported by the mothers.

Table I presents ORs for the intakes of food groups at ages 10 and 3 and during weaning as reported by mothers. Consumption of salted fish (excluding Guandong salted fish) was associated with slightly elevated risks at age 10 and during weaning. Consumption of Guandong salted fish (moldy and firm combined) was very rare (data not shown); only 1 case was exposed, and intake was reported by the mother for ages 10 and 3, during weaning and while she was breast-feeding. Consumption of cured and smoked meats at ages 10 and 3 was not associated with risk. Cured meats were not consumed during weaning, and only 1 case and 2 controls consumed smoked meats. Salted egg intakes at ages 10 and 3 were not associated with risk (data not shown); however, salted egg consumption during weaning was associated with a 90% increased risk (95% CI 0.7–5.3) based on 10 cases and 9 controls.

High consumption of fermented soybean products was associated with non-significantly decreased risks of NPC for intakes at ages 10 and 3 and during weaning. High intake of fresh soybean products was associated with a significantly decreased risk at age 10 and a decreased risk at age 3. Consumption during weaning was not associated with risk.

Preserved vegetable intake was not associated with risk for any childhood diet period. However, fresh vegetables (1 serving/week) were associated with decreased risks for intakes at ages 10 and 3 and during weaning. Fruit intake at ages 10 and 3 was not associated with risk. Intake more than once per week during weaning was associated with a non-significantly elevated risk based on 7 cases and 11 controls.

Nitrosamines and nitrite. We compared nitrite and nitrosamine intakes at age 10 as reported by the subject and the mother. For both mothers' and subjects' reports of intake of nitrite and nitrosamines from soybean products, ORs were <1, though not significant for intake above the lowest quartile (mothers' reports Table II). The mothers' reports of age 10 intake of nitrite and nitrosamines from other foods were associated with 2- to 3-fold elevated risks of NPC for the third and fourth quartiles of intake. Intake as reported by the subject was not associated with risk (highest *vs.* lowest quartile, nitrite OR = 1.1, 95% CI 0.5–2.5; nitrosamines OR = 1.2, 95% CI 0.5–2.4).

Intake of nitrite from soybean products at age 3 was not associated with risk; however, intake of nitrite from soybean products during weaning was associated with decreased risk of NPC compared to those with no intake (Table II). ORs were elevated for intakes of nitrite from other products for both age 3 and weaning diets. The results for nitrosamine intake at age 3 and during weaning were similar to those for age 10 intake as reported by the mother. Decreased risk was observed for nitrosamine intakes from soy products above the lowest quartile, whereas risks were elevated for intakes of nitrosamines from other dietary sources.

Adjustment for vegetable intake increased the magnitude of the ORs for nitrite and nitrosamine intake from non-soy products slightly. There was no evidence of effect modification by level of intake of fruits, vegetables or fresh soybean products.

Mother's diet during breast-feeding

The mother's diet during breast-feeding showed no strong or significant positive associations with risk of NPC in her child (data not shown). Inverse associations were observed with increasing intake of fermented soybean products (highest quartile OR = 0.7, 95% CI 0.3–1.6), fresh soy products (OR = 0.6, 95% CI 0.2–1.5), preserved vegetables (OR = 0.6 , 95% CI 0.2–1.5), fresh vegetables (OR = 0.3, 95% CI 0.1–1.5) and fruit (OR = 0.5, 95% CI 0.2–1.5).

The mother's intake of nitrite from soybean products while breast-feeding was associated with a non-significantly decreased risk of NPC (highest *vs.* lowest quartile OR = 0.7, 95% CI 0.3–1.7), whereas intake of nitrite from other foods was not associated with risk. Intake of nitrosamines from soybean products above the lowest quartile was associated with decreased risk of NPC (highest *vs.* lowest quartile OR = 0.6, 95% CI 0.2–1.4). For the highest intake of nitrosamines

TABLE I – ODDS RATIO (OR) AND 95% CONFIDENCE INTERVAL (CI) FOR NPC WITH CONSUMPTION OF FOOD GROUPS AS A CHILD AT AGE 10, AGE 3 AND DURING WEANING[1]

Frequency (g/week)	Age 10				Frequency (g/week)	Age 3				Frequency (g/week)	Weaning			
	Cases	Controls	OR[2]	(CI)		Cases	Controls	OR[2]	(CI)		Cases	Controls	OR[2]	(CI)
Salted fish[3]														
0	50	75	1.0		0	65	79	1.0		0	74	98	1.0	
>0	32	35	1.5	(0.8–2.8)	>0	21	23	1.0	(0.5–2.1)	>0	10	7	1.7	(0.6–5.1)
Cured meat														
0	53	67	1.0		0	76	87	1.0		0	89	112	1.0	
>0	36	46	1.1	(0.6–2.0)	>0	16	22	0.8	(0.4–1.8)	>0	0	0		
Smoked meats														
0	76	95	1.0		0	84	100	1.0		0	88	110	1.0	
>0	15	19	1.1	(0.5–2.4)	>0	8	10	1.2	(0.4–3.3)	>0	1	2	2.2	(0.2–27.6)
Fermented soybean products														
4.1	34	29	1.0		0	15	21	1.0		0	38	39	1.0	
4.1–10.0	17	28	0.4	(0.2–0.9)	>0.1–5.1	31	32	0.9	(0.4–2.4)	0.01–.35	9	18	0.5	(0.2–1.4)
10.1–29.0	16	27	0.4	(0.2–0.9)	5.2–20	22	30	0.6	(0.2–1.5)	0.36–3.0	22	28	0.6	(0.3–1.3)
>29.0	26	31	0.5	(0.2–1.2)	>20	25	28	0.7	(0.3–1.9)	>3.0	21	28	0.6	(0.3–1.3)
Fresh soybean products														
<0.5	32	28	1.0		<0.04	21	26	1.0		0	57	71	1.0	
0.5–1.0	21	27	0.6	(0.2–1.2)	0.04–0.5	34	35	1.3	(0.6–2.8)	>0	27	33	1.1	(0.5–2.1)
1.1–1.5	26	23	0.9	(0.4–2.0)	0.6–1.0	24	25	1.2	(0.5–2.7)					
>1.5	14	36	0.4	(0.2–1.0)	>1.0	11	24	0.7	(0.3–1.7)					
Preserved vegetables														
0	20	25	1.0		0	42	50	1.0		0	63	87	1.0	
0.07–3.7	24	37	0.7	(0.3–1.7)	0.05–2.7	12	19	0.6	(0.2–1.4)	>0	24	25	1.3	(0.6–2.5)
3.8–14.7	32	35	0.9	(0.4–2.1)	2.8–11	23	22	1.2	(0.6–2.5)					
>14.7	13	16	0.9	(0.3–2.6)	>11	11	12	0.9	(0.3–2.4)					
Servings/week, vegetables														
0–1	14	7	1.0		0–1	26	19	1.0		0–	24	22	1.0	
>1	77	107	0.4	(0.1–1.0)	>1	61	91	0.5	(0.3–1.1)	0.1–1	16	20	0.8	(0.3–2.0)
										>1	44	69	0.6	(0.3–1.3)
Servings/week, fruits														
<0.12	22	28	1.0		0	25	29	1.0		<0.1	51	64	1.0	
0.12–0.5	23	22	1.1	(0.5–2.6)	0.01–0.2	17	22	0.8	(0.3–1.8)	0.1–0.8	13	13	1.6	(0.6–3.8)
0.6–3.5	32	31	1.5	(0.7–3.4)	0.3–1	36	35	1.3	(0.6–2.8)	0.9–1.9	13	18	1.3	(0.5–3.1)
>3.5	13	25	0.9	(0.3–2.3)	>1	9	19	0.9	(0.3–2.6)	>1.9	7	11	2.1	(0.6–7.4)

[1]Childhood intake as reported by the mother for ages 10 and 3 and during weaning.–[2]ORs were adjusted for age, gender and ethnicity. –[3]Excluding Guandong salted fish.

from other products there was a slightly elevated risk (highest *vs.* lowest quartile OR = 1.4, 95% CI 0.5–3.6) but no association at lower intake levels. Risks were similar across levels of intake of fruits, vegetables and fresh soybean products.

DISCUSSION

We found an increased risk of NPC with higher childhood intake of nitrite and nitrosamines overall and when the sources of intake were meat, fish and preserved vegetables. Nitrite is not carcinogenic, but it reacts with secondary amines and amides to form carcinogenic nitrosamines and nitrosamides in the body, a process called endogenous nitrosation (National Academy of Sciences, 1981). Higher childhood intakes of nitrite and nitrosamines from soybean products were generally associated with a nonsignificantly decreased risk of NPC in our study. Compounds in soybeans may have anti-carcinogenic properties (Messina and Barnes, 1991), and a few animal studies indicate that they may inhibit endogenous nitrosation (Mokhtar *et al.*, 1988; Fitzsimons *et al.*, 1989). Endogenous nitrosation may be the major source of exposure to *N*-nitroso compounds (Shepard and Lutz, 1989); thus, consumption of nitrosation inhibitors may play an important role in reducing exposure to *N*-nitroso compounds.

Salted fish intake has been strongly associated with the risk of NPC in previous studies in Chinese populations. We observed elevated risks for childhood and weaning intakes of salted fish but not for adult intake. The elevated risks we observed were not significant and were much lower than those found in most previous studies in China (Yu *et al.*, 1986, 1988, Yu *et al.*, 1989*a*; Ning *et al.*, 1990; Zheng *et al.*, 1994) but consistent with one previous study in Taiwan (Chen *et al.*, 1988). Some studies have also observed positive associations for weekly or daily intake as an adult (Armstrong *et al.*, 1983, 1998; Yu *et al.*, 1986, 1989*a*), but another did not (Ning *et al.*, 1990).

Our analysis of specific foods and some food groups was limited by the small numbers of individuals with high intake. Salted fish, cured meat and smoked meat consumption was uncommon during childhood. Other preserved foods were eaten more frequently, but with few exceptions, consumption was not associated with increased risk. Few studies of NPC have reported risk estimates for intakes of preserved foods other than salted fish. Chinese preserved or dried foods that were associated with risk in 1 or more study (Yu *et al.*, 1988, 1989a; Ning *et al.*, 1990; Zheng *et al.*, 1994; Lee *et al.*, 1994; Armstrong *et al.*, 1998) included salted eggs, salted shrimp paste, salted leafy vegetables, salted mustard greens, pickled vegetables, dried fish, salted soybeans, fermented soybean pastes, moldy bean curd and melon seeds. In contrast with these studies, we found no association for preserved vegetable products and an inverse association with intake of preserved or moldy soybean products. Salted egg consumption during weaning was

TABLE II – ODDS RATIO (OR) AND CONFIDENCE INTERVAL (CI) FOR NPC WITH LEVELS OF DIETARY INTAKE OF NITRITE AND NITROSAMINES DURING CHILDHOOD AT AGE 10, AGE 3 AND DURING WEANING[1]

Index	Age 10		Age 3		Weaning	
	OR[2]	(95% CI)	OR[2]	(95% CI)	OR[2]	(95% CI)
Nitrite from soybean products						
Q1 (low)	1.0		1.0		1.0	
Q2	0.6	(0.2–1.3)	1.6	(0.6–3.9)	0.6	(0.2–1.5)
Q3	0.5	(0.2–1.1)	1.0	(0.4–2.5)	0.8	(0.3–1.8)
Q4 (high)	0.6	(0.3–1.4)	1.2	(0.4–3.0)	0.7	(0.3–1.4)
Nitrite from other foods						
Q1 (low)	1.0		1.0		1.0	
Q2	1.7	(0.6–4.9)	2.6	(0.5–12.0)	2.6	(1.0–7.2)
Q3	3.5	(1.3–9.5)	2.5	(0.5–11.5)	4.9	(1.1–21.6)
Q4 (high)	2.0	(0.7–6.0)	2.1	(0.4–10.2)	2.8	(0.6–13.0)
Nitrosamines from soybean products						
Q1 (low)	1.0		1.0		1.0	
Q2	0.5	(0.2–1.1)	0.7	(0.3–1.6)	0.6	(0.2–1.7)
Q3	0.5	(0.2–1.2)	0.7	(0.3–1.6)	0.7	(0.3–1.5)
Q4 (high)	0.6	(0.3–1.4)	0.7	(0.3–1.8)	0.8	(0.4–1.8)
Nitrosamines from other foods						
Q1 (low)	1.0		1.0		1.0	
Q2	2.1	(0.9–5.4)	2.5	(1.0–6.7)	2.3	(0.9–6.1)
Q3	3.0	(1.2–7.3)	2.5	(1.0–6.4)	1.5	(0.5–4.0)
Q4 (high)	2.2	(0.8–5.6)	2.6	(1.0–7.0)	3.9	(1.4–10.4)

[1]Childhood and weaning diet as reported by the mother.–[2]ORs adjusted for age, gender, ethnicity and vegetable intake.

associated with an increased risk of NPC in our study, though the association was less than the 5-fold risk previously observed (Yu *et al.,* 1988). Our finding of an inverse relationship with fresh vegetable intake is consistent with previous results (Yu *et al.,* 1989a; Ning *et al.,* 1990; Zheng *et al.,* 1994; Armstrong *et al.,* 1998). Consumption of soybean products during childhood also was inversely associated with risk, a relationship that to our knowledge has not been reported previously.

The fact that we observed weak associations with childhood consumption of salted fish and little or no association with other preserved foods may be the result of several factors. In recent years, consumption of commercially produced salted fish and other preserved foods has increased and consumption of home-preserved foods with higher nitrite and nitrosamine levels has declined (W.-H. Pan, personal communication). To some extent, risk estimates from earlier studies of NPC may reflect higher exposure levels in the past. Further, regional variations in the preparation of salted fish and other preserved foods might explain the differences across studies. For example, Guandong salted fish, which has the highest reported levels of nitrosamines due to the method of preparation, is not commonly eaten in Taiwan. Other methods of salted fish preparation that result in lower nitrosamine levels are more common. Likewise, differences in the preparation methods for other preserved and fermented foods may result in lower levels of these contaminants in Taiwanese foods compared to other areas. The lack of information on levels in many Taiwanese foods did not allow us to evaluate this possibility directly.

Despite the fact that we found few positive associations between childhood intake of individual preserved foods and risk of NPC, we found an increased risk with higher estimated intake of nitrite and nitrosamines during childhood. Our results illustrate the importance of considering all dietary sources of intake because the intake of individual foods and food groups may not adequately characterize the total dietary intake of these compounds.

We observed a positive association with age 10 intake of nitrosamines and nitrite from non-soy foods as reported by mothers; however, there was no association with intake reported by subjects. Correlations between the subjects' and mothers' reported intakes were low to moderate (Pearson's correlation coefficients of 0.1 to 0.4) for most foods and food groups and similar for cases and controls. Subjects' reported intake at age 10 was highly correlated with their adult intake for most foods. The dietary questionnaire included questions about adult dietary intakes prior to the questions about age 10 intakes; thus, recall of the adult diet may have influenced reporting of age 10 intake. Because adult intake of these compounds was not associated with risk, the similarities between adult and age 10 intakes may explain the lack of association among self-respondents.

Two previous studies of NPC compared mothers' and subjects' reports of consumption of salted fish at age 10 and found similar 2-fold increased risks (Yu *et al.,* 1986, 1989*a*). Most other studies relied on mothers or other family members for information about early childhood intake and generally found strong positive associations with consumption of salted fish during weaning or early childhood (Armstrong et al., 1983; Yu *et al.,* 1988; Ning *et al.,* 1990; Zheng *et al.,* 1994). Few studies have evaluated the validity of mothers' reports of their children's diet. Byers *et al.* (1993) assessed the validity of parental reports of their children's fruit and vegetable intake by comparing intake levels reported by parents to serum nutrient levels in the children. Correlations between parental reported intake and serum levels were good and similar in magnitude to those observed in comparisons between self-reported intake and serum levels.

Dietary intakes of nitrosamines, nitrite, and nitrate have been evaluated previously in studies of gastro-intestinal tract cancers using an approach similar to ours (Rogers *et al.,* 1993; Risch *et al.,* 1985). The limitations of this approach of assigning values to foods based on published reports include the limited data on levels of these compounds in some foods and the variation in levels across studies. Comprehensive surveys of nitrosamines in foods have been conducted in several countries, and most foods commonly consumed in Western countries have been analyzed for volatile *N*-nitroso compounds. Asian foods have not been studied to the same extent, but the existing studies indicate that the levels can be substantially higher and detection more frequent compared to Western foods. Most studies of Asian foods have focused on foods associated with risk of NPC or other cancers. Data for other foods and for nitrite and nitrate are limited. Variation in levels of nitrite and nitrosamines due to different preparation methods and the lack of specific data for some Taiwanese foods most likely led to some misclassification in our assignment of exposure categories to foods. Misclassification of exposure would be non-differential by case and control status and, thus, under most circumstances would attenuate risk estimates (Correa *et al.,* 1995).

Only the volatile *N*-nitroso compounds are easily analyzed (Tannenbaum, 1983), and most studies have measured only a few (Hotchkiss, 1989). Because of the technical difficulties in their analysis, the non-volatile *N*-nitroso compounds have not been routinely measured in foods, though some data suggest that they occur at a greater frequency and at higher levels than the volatile compounds (Hotchkiss, 1989). We based our categorization of nitrosamine levels in foods on the compounds with the highest measured level if multiple nitrosamines were analyzed. However, for many foods, only 1 compound, dimethylnitrosamine (DMNA), was measured. Misclassification of exposure may occur if DMNA levels are not a good surrogate of nitrosamine exposures relevant to risk. Misclassification of the relevant etiological nitrosamine and nitrite levels would tend to dilute risk estimates and may also account for the lack of a clear dose response with increasing levels of intake.

Experimental animal studies support an etiological role for consumption of salted fish in NPC oncogenesis (Yu *et al.,* 1989*b*;

Huang *et al.,* 1978). The evidence that nitrosamines may be etiologically important agents in NPC development comes from several sources. Diethylnitrosamine and DMNA have been tested in over 20 species including non-human primates and found to induce tumors in all of them. The nasal cavity is a common site for tumor induction in rats fed nitrosamines. The carcinogenic potency of nitrosamines is generally the greatest for the oral route of administration when the compound is given in many small doses over time (Lijinsky and Taylor, 1977). This route and pattern of exposure characterize dietary intake. CYP2E1, which metabolizes nitrosamines to their reactive carcinogenic forms, is expressed in the nasal epithelium of humans and other animals. In this study, individuals homozygous for the c2 allele of the *CYP2E1* gene, which may result in higher gene expression, had an increased risk of NPC (Hildesheim *et al.,* 1997).

The possibility that other components in salted fish and preserved foods might be the etiologically important agents cannot be ruled out. Several studies have demonstrated that another class of compounds isolated from salted fish was capable of inducing Epstein-Barr virus, a known risk factor for NPC (Hildesheim and Levine, 1993). For another class of compounds to explain the association we observed, the levels of nitrosamines and these compounds would have to be positively correlated across many different foods.

Previous epidemiological studies have evaluated individual preserved foods as risk factors for NPC and other cancers; however, most previous studies have not estimated intakes of nitrite and nitrosamines across all foods. Our results further support the hypothesis that intake of nitrosamines and nitrosamine precursors during childhood is a risk factor for the development of NPC.

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APPENDIX – INDIVIDUAL FOODS INCLUDED IN EACH FOOD GROUP FOR SUBJECT'S DIET AT DIFFERENT AGES AND FOR MOTHER'S DIET WHILE BREAST-FEEDING

	Adult	Age 10	Age 3	Weaning	Breast-feeding
Cured or salted meats and duck	X	X	X	X	X
Smoked meats, chicken and fish	X	X	X	X	X
Processed fish and seafood (including sauces)					
Wuzi, tiozi and other fish with edible bones	X	NA[1]	NA	NA	NA
Dried, shelled shrimp	X	NA	NA	NA	NA
Sacha sauce	X	NA	NA	NA	X
Seafood sauce, shrimp sauce, fish sauce, oyster sauce, other sauces	X	NA	NA	NA	X
Hot pepper sauce	X	X	NA	NA	X
Guandong moldy fragrant salted fish	X	X	X	X	X
Guandong firm salted fish	X	X	X	X	X
Other types of salted fish	X	X	X	X	X
Preserved eggs	X	X	X	X	X
Salted eggs	X	X	X	X	X
Fermented soybean products					
Strong-smelling preserved bean curd	X	X	X	X	X
Fermented bean curd	X	X	X	X	X
Fermented rice	X	X	X	X	X
Thick broad bean sauce, fried bean sauce (sweet sauce of fermented flour)	X	X	X	NA	X
Soy sauce (extra added, soaked)	X	X	X	NA	X
Fermented soybeans	X	X	X	X	X
Miso	X	X	X	NA	X
Fresh soybean products					
Tofu	X	X	X	X[2]	X
Fried tofu, bean curd skin, dried bean strips and other bean curd products	X	X	X	X[2]	X
Soybean milk, bean flowers	X	X	X	X[2]	X
Soybeans	X	X	X	NA	X
Preserved vegetables					
Dried radish (turnip), pickled cabbage, preserved mustard, rutabaga pickles, other pickled vegetables	X	X	X	X	X
Fermented mustard greens	X	NA	NA	NA	NA
Other pickled, fermented, molded food	X	NA	NA	NA	NA
High-nitrate vegetables					
Spinach, water convolvulus, mustard greens, cauliflower, potato leaves, rape, crown daisy, chrysanthemum, radish greens and other dark green vegetables	X	NA	NA	NA	NA

[1]NA, not asked.–[2]Asked as 1 question: soybean milk, soybean flowers, tofu and other soybean products.