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N-Nitroso Compounds: Assessing Agreement between Food Frequency Questionnaires and 7-day Food Records

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Abstract

N-nitroso compounds are recognized as important dietary carcinogens. Accurate assessment of *N*-nitroso intake is fundamental to advancing research regarding its role with cancer. Previous studies have not used a quantitative database to estimate their intake. To address this gap a database of *N*-nitroso values was developed in conjunction with an existing food frequency questionnaire (FFQ). The purpose of this paper is to report on the relative validity of the FFQ instrument modified to estimate *N-n*itroso compounds. Intake estimates of 23 *N*-nitroso compounds from FFQ were compared with those from seven days of food records (7DFR) in a cross-sectional study conducted from January 2005 through June 2006. A sample of 98 healthy adult subjects (50.42 ± 12.84 years) completed a FFQ and then recorded foods and beverages consumed (7DFR). Crude and energy-adjusted *N*-nitroso compounds intakes were significantly higher in the FFQ than the 7DFR($P < 0.001$). Spearman correlations for crude and energy-adjusted *N-n*itroso intakes ranged from 0.004 to 0.48. By tertiles of *N*-ntiroso compounds, there was moderate agreement (Kappa >0.30) for five compounds. Higher estimates of *N*-nitroso compounds by FFQ was explained by a greater proportion of subjects who reported eating foods high in *N*-nitroso compounds on FFQ than reported on 7DFR. The modified FFQ with *N-n*itroso values is a useful tool for assessing *N*-nitroso intakes relative to a group, and captures all food items with *N*-nitroso compounds including those foods with high concentrations and eaten sporadically.

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Keywords

dietary methodology; validation; dietary carcinogens

The potential role of dietary intake of *N*-nitroso compounds and their precursors in the development of cancer is of growing interest (1-3). *N*-nitroso compounds risk for human cancer is supported by animal experiments (1,2), and mechanisms describing DNA damage (4). Epidemiological studies evaluating the role of *N*-nitroso compounds have shown a positive association with stomach and upper gastro-intestinal tract cancers (5-8) and brain cancer (3, 9-13), whereas others found no association (14-17).

The formation of these compounds in foods (3) occurs when oxides of nitrogen react with amino groups, yielding *N*-nitrosamines or *N*-nitrosoamides. Foods with the highest concentrations of *N*-nitroso compounds are those cured with nitrate and/or nitrite (cured meat), pickled, stored under humid conditions, smoked in air saturated with nitrogen, or dried at high temperatures (i.e. protein-containing foods such as beer ingredients, nonfat dry milk, cooked bacon, or dried meats) (18,19).

Lack of compiled data on *N-n*itroso compounds in foods into a database, prevented past dietary epidemiological studies to accurately measure intakes of *N*-nitroso compounds since exposure was surrogate or incomplete measures. To address this gap, a database of *N*-nitroso compounds was created and linked to an existing food frequency questionnaire (FFQ). The purpose of this paper is to report on the relative validity of the FFQ instrument modified to estimate *N*-nitroso compounds.

Methods

Study Population

The research was a cross-sectional study design, conducted January 2005 to June 2006. Subjects were recruited from a database of healthy controls who had completed data collection in studies at M.D. Anderson Cancer Center, and from the community using Institutional Review Board approved announcements. Subjects did not have a previous diagnosis of any cancer type and were frequently-matched to the cancer cases on age (± 5 years), gender, and ethnicity. Subjects were interviewed in person and completed a FFQ, and then were invited to participate in current study requiring completion of 7-day food records. All subjects gave their consent to participate in the study, approved by the Institutional Review Board of the Human Subjects Protection Program at The University of Texas, M.D. Anderson Cancer Center and Baylor College of Medicine.

Seven Day Food Records

The 7-day food record was chosen as the reference method. The study nutritionists gave subjects both verbal and written instructions for completing a 7-day food record, including detailed information on the amounts consumed and detailed food descriptions. Foods reported on the 7-day food records were coded and analyzed using Nutrition Data System for Research (NDS-R) software (version 2005, Nutrition Coordinating Center, University of Minnesota, Minneapolis).

The assignment of values for *N*-nitroso compounds to foods in the 7-day food records followed standards of practice on food composition data sources (20-22) whereby research database foods (and corresponding *N*-nitroso values of μg/100 g) are matched to line items of each food listed in the food record. Concentration was multiplied by grams of food consumed and yielded the *N*-nitroso intake for each food, *N*-nitroso values were summed from all food sources per day, and a mean of 7 days was computed for each subject.

Food Frequency

The food frequency questionnaire, a modified version of the National Cancer Institute's Health Habits and History Questionnaire (23), was used to collect dietary data. The questionnaire includes a semi-quantitative food frequency list made up of food and beverage items, ethnic foods commonly consumed in the Houston area, and an open-ended section. Nutrient intake was calculated using the DIETSYS+Plus version 5.9 dietary analysis program (Block Dietary Data Systems, 1999, Berkeley, Calif). The DIETSYS+Plus database was updated using release 16-1 of the United States Department of Agriculture National Nutrient Database for Standard Reference (24). Nutrient values were estimated as needed from appropriate recipes found in the Continuing Survey of Food Intakes by Individuals, 1994-1996, 1998 for multi-ingredient dishes not available in release 16-1 (25).

The assignment of values for *N*-nitroso compounds to foods in the FFQ was completed (20-22) whereby research database foods are matched to line items of each food listed in the FFQ. The 205 food items in the modified FFQ were assigned *N*-nitroso values by matching foods using a direct match or imputation method (21). For both FFQ and 7DFR, the output variables were 23 *N*-nitroso compounds, gram intake for selected food groups of *N*-nitroso sources, and macronutrients provided by nutrient analysis software.

Data and Statistical Procedures

A sample size of 100 allowed a power of 0.9 to detect desirable differences between methods. This sample size of 100 also conformed to the range (100-200) recommended by Willett and Lenhart (26) needed for a validation study for dietary questionnaires.

Statistical analyses were conducted using SAS statistical software, (version 9.1, 2003, Statistical Analysis Systems, Cary, NC). Means and standard deviations were calculated for demographic variables and macronutrient intakes. *N*-nitroso compound intakes are reported as medians (25^{th} and 75^{th} percentiles). Energy-adjusted intakes were computed using the residual method (27). The Wilcoxon sign rank test was used to determine whether *N*-nitroso intakes (crude or energy-adjusted) of FFQ and 7DFR were significantly different. The joint classification (%) of the participants in each tertile for 7DFR vs FFQ for each compound was calculated. Specifically, how many participants in the lowest and highest tertiles of N-Nitroso compound intake by 7DFRs, (as the reference method), were classified in the same tertiles by FFQ. A weighted kappa was calculated which accounts for agreements and disagreements (28).

Willett (27) argued that standard summary statistics prevent detection whether a dietary questionnaire discriminates among persons because of omissions or additions of foods or differences in portion size. Accordingly, the proportion of subjects who reported eating the high N-nitroso food source (yes/no) by each method was computed, and agreement of subjects reporting intake of food sources on both methods was tested using the McNemar X^2 test statistic for paired data. All statistically significant differences were based on a $P < 0.05$. All tests were two-tailed.

Results and Discussion

The subjects (n = 98) who participated in the study had a mean age 50.42 ± 12.84 years, mean body mass of index 26.51 ± 5.94 kg/m^2, and a majority (73%) were white and female (61%). Based on 7DFR, subjects reported mean intakes of 1727.25 ± 512.97 kcal, 68.67 ± 24.37 g. fat and 73.02 ± 25.17 g protein, and based on FFQ mean intakes of 2370.53 ±1369.20 kcal, 91.96 ± 54.93 g fat, and 96.53 ± 53.35 g protein. An Appendix Table lists full chemical names and corresponding abbreviation for each *N*-nitroso compound.

In Table 1, the median (25^{th} and 75^{th} percentiles) of the intake for *N*-nitroso compound as reported by FFQ and 7DFR are shown. Crude and energy adjusted *N*-nitroso intakes were significantly higher in the FFQ than for the 7DFR, energy adjustment did not change the estimates. Also shown in Table 2 are *P values* for Wilcoxon sign rank and the Spearman correlation coefficients for crude and energy-adjusted *N*-nitroso intakes estimated from the FFQ and 7DFR. Spearman correlations for ranking by the two methods ranged from -0.04 to 0.48, and highest correlation between crude estimates were for six compounds (0.40 to 0.48).

The Bland Altman plots (29) for the differences vs averages were completed all *N*-nitroso compounds (Data not shown). Wide limits of 95% agreement and positive slopes were driven by higher FFQ intakes (i.e. increasing mean difference in intake vs. average of two methods). Findings for the joint classification of energy adjusted *N*-nitroso intake by tertiles of FFQ vs. 7DFR (data not shown) revealed weighted kappas of nearly 0.30 for five compounds, indicating moderate agreement for NAA, NTCA, NDMA, N03, and N02. The corresponding percentage agreement for these kappas was at least 50% of both high and low tertiles for these compounds by FFQ agreed with the benchmark classification by the 7DFR. The greatest percentage agreement for being ranked in the lowest tertile by both methods was 56% for NDMA. The greatest percentage agreement was 60% for being ranked in the highest tertile by both methods was for NAA.

A comparison of individuals reporting intake of high *N*-nitroso food sources of (Table 2) ham, wine, hot dog, bacon, sausage, beer and beef showed a greater proportion reported on FFQ than for 7DFR. Pairwise comparisons for individuals reporting intake of the food groups showed statistically significant differences for wine, bacon, sausage and hot dog (corrected X^2, McNemar statistic), which confirms individuals reported more frequently on FFQ than 7DFR. The cumulative occurrence of this finding demonstrated that 37% percent of individuals who reported consumption of two or more high *N*-nitroso food groups on FFQ, did not report these same groups on the 7DFR.

Food frequency questionnaires have been previously used in large scale epidemiological studies to obtain dietary exposure data (27), but have not measured N-nitroso compounds. In this study, *N*-nitroso compound intake was measured by FFQ and 7DFR in a sample of healthy subjects. Crude and energy adjusted *N*-nitroso values were higher in the FFQ than the 7DFR. This study also provided intake estimates of the *N*-nitroso compounds, where median *N*-nitroso intakes ranged from <0.001 μg/d to 15 μg/d, with skewed distributions.

Spearman's rank correlations on *N*-nitroso compounds from the present study are similar to those observed by Brunner and colleagues (30) in a study of 835 adult men and women in England, measuring agreement on nutrient intakes between 7-day diet diary and FFQ. For the women in their study the value ranged from 0.22 for energy to 0.86 for alcohol, median of 0.37, comparable with our overall median of 0.32. Correlations of 0.60 to 0.80 indicate moderate agreement, and > 0.81 close agreement. Brunner's group also examined agreement across quartiles of intake: exact quartile agreement achieved ranged from 29% for carotenes to 65% for alcohol, with a median of 35%; exact agreement in the present study by tertiles ranged from 0 to 50%. Willett and colleagues (26) reported a comparison of nutrient data from an FFQ versus a diet diary. Pearson correlation coefficients ranged from 0.18 for protein to 0.52 for sucrose with a median of 0.32.

To further explain the collective findings of higher intakes measured by the FFQ, an analysis was conducted whether foods high in *N*-nitroso compounds were uniformly reported on FFQ vs. 7DFR. It is expected that *N*-nitroso compounds are contributed by definite food sources eaten episodically, (i.e., several non-consumption days, with large day to day variation). A greater proportion of individuals reported both higher frequencies and intake of foods high in *N*-nitroso foods on FFQ than by 7DFR. The 7DFR may not be sufficient to capture the variability of high *N*-nitroso food sources, consumed infrequently. However, complex statistical procedures are under development by the NCI and others (31,32) to estimate intake of episodically consumed foods from few 24-hour recalls. Hence, when the focus is on a compound contributed by distinct food sources (for *N*-nitroso compounds) food consumption episodes may be captured by a FFQ. Therefore, these results support use of a FFQ questionnaire to categorize individuals, which coincide with the goal of using questionnaires to classify individuals on a food component relative to the group rather than capacity to measure group means (27). These results also suggest the need for more development on questionnaires targeted for *N*-nitroso compounds, similar to specialized questionnaires underway related to meat preparation and heterocylic amines (33,34).

The major limitation is that the *N*-nitroso database is not complete. The database was created with all *N-n*itroso compounds on the basis of all available literature, yet many foods and compounds have not been assayed. However, concentration of *N*-nitroso compounds depends on preparation techniques and other environmental factors.

Conclusions

Quantifying the dietary intake of *N-n*itroso compounds is relevant for research on the relationship of diet with cancer. *N-n*itroso values linked to a commonly used FFQ permitted estimates of these compounds. The FFQ captured sporadic intakes, ranked individuals relative to the group, and a greater proportion of individuals reported consumption of foods high in *N-n*itroso compounds (i.e., ham, wine, hot dog, bacon, sausage, and beef) on FFQ than 7DFR. The FFQ modified with *N-n*tiroso values and validated in the present research will permit future analyses into the relationships between diet and cancer. Dietitians need to be aware of the potential risk of *N-N*itroso compounds, and efforts to assess their intake using a valid instrument are an essential first step. However, much work remains to be done to estimate intake of *N-N*itroso compounds and develop strategies for prevention of their risk.

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Appendix

Appendix Table

N-Nitroso Compound List

Abbreviation	Compound Name
NAA	N-Nitrosoamino acids
NDBA	N-Nitrosodibutylamine

Abbreviation	Compound Name
NDBZA	N-Nitrosodibenzylamine
NDEA	N-Nitrosodiethylamine
NDMA	N-Nitrosodimethylamine
NDPA	N-Nitrosodipropylamine
NHMT	N-nitroso-2-(hydroxymethyl)thiazolidine
NHMTCA	N-nitroso-2-hydroxymethylthiazolidine-4-carboxylic acid
NHPRO	N-nitroso-4-hydroxyproline
NMAMBA	N-nitroso-N-(1-methylacetonyl)-3-methylbutylamine
NMEA	N-Methyl-N-ethylnitrosamine
NMOCA	N-nitroso-5-methyloxazolidine-4-carboxylic acid
NMOR	N-Nitrosomorpholine
NMTCA	N-nitroso-2-methylthiazolidine-4-carboxylic acid
NPIP	N-Nitrosopiperidine
NPYR	N-Nitrosopyrrolidine
NPRO	N-nitrosoproline
NSAR	N-Nitrososarcosine
NTCA	N-nitrosothiazolidine-4-carboxylic acid
NTHZ	N-nitrosothiazolidine
NTHZCA	N-nitrosothiazolidine carboxylic acid and related homologues
NO3	Nitrate
NO2	Nitrite

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Table 1

Median daily N-Nitroso compound intakes and correlation between N-Nitroso compound intakes reported on 7-Day Food Records and Food Frequency Questionnaire

N-Nitroso Compound	**7-Day Food Record** [a]	**Food Frequency Questionnaire** [a]	***P* value**[b]	R^c	R^d
	Median (25th, 75th percentiles)				
NAA **(μg)**	0.101 (0.056, 0.252)	0.146 (0.078, 0.237)	0.04	0.48	0.31
NDBA **(μg)**	1.978 (0.983, 4.260)	3.65 4 (1.796, 5.435)	0.001	0.37	0.22
NDBZA **(μg)**	0.000 (0.000, 0.449)	0.675 (0.197, 1.134)	<0.0001	0.24	0.18
NDEA **(μg)**	0.086 (0.067, 0.128)	0.168 (0.112, 0.232)	<0.001	0.10	0.16
NDMA **(μg)**	0.545 (0.275 1.618)	1.038 (0.589, 1.430)	0.0148	0.43	0.19
NDPA **(μg)**	0.157 (0.087, 0.234)	0.122 (0.088, 0.192)	0.274	0.30	0.35
NHMT **(μg)**	0.001 (0.000, 0.003)	0.004 (0.001, 0.007)	0.002	0.24	0.18
NHMTCA **(μg)**	32.896 (13.181, 48.673)	23.489 (11.485, 41.201)	0.159	0.44	0.42
NHPRO **(μg)**	0.179 (0.000, 0.483)	0.705 (0.271, 1.336)	<0.0001	0.33	0.28
NMAMBA **(μg)**	0.013 (0.009, 0.020)	0.027 (0.016, 0.041)	<0.0001	0.38	0.27
NMEA **(μg)**	0.001 (0.001, 0.002)	0.002 (0.001, 0.004)	<0.0001	0.38	0.26
NMOCA **(μg)**	0.000 (0.000, 0.142)	0.214 (0.071, 0.400)	<0.0001	0.26	0.18
NMOR **(μg)**	0.013 (0.005, 0.042)	0.046 (0.025, 0.090)	<0.0001	0.29	0.28
NMTCA **(μg)**	0.057 (0.000, 0.184)	0.250 (0.100, 0.460)	<0.0001	0.14	0.20
NPIP **(μg)**	0.012 (0.008, 0.019)	0.031 (0.020, 0.047)	<0.0001	0.07	0.10
NPYR **(μg)**	0.166 (0.101, 0.266)	0.460 (0.317, 0.651)	<0.0001	0.38	0.37
NPRO **(μg)**	1.019 (0.603, 1.625)	2.763 (2.007, 4.084)	<0.0001	0.23	0.21
NSAR **(μg)**	0.000 (0.000, 0.040)	0.060 (0.020, 0.160)	<0.0001	0.05	-0.004
NTCA **(μg)**	6.990 (3.960, 14.054)	15.813 (8.134, 24.616)	<0.0001	0.39	0.37
NTHZ **(μg)**	0.254 (0.142, 0.391)	0.428 (0.255, 0.641)	<0.0001	0.44	0.42
NTHZCA **(μg)**	0. 000 (0.000, 4.019)	0.265 (0.000, 1.300)	0.01	0.40	0.16
N03 (mg)	42.05 (25.56, 59.54)	59.74 (39.92, 91.54)	<0.0001	0.31	0.25
NO2 (mg)	1.19 (0.57, 1.89)	1.85 (1.10, 2.87)	<0.0001	0.47	0.33

[a] Subjects (n = 98) were aged 50.42 ± 12.84 yrs at data collection

[b] *P* values for Wilcoxon sign rank rest comparing medians reported on the Food Frequency Questionnaire and 7-Day Food Record

[c] Spearman correlation coefficients between crude intakes reported on the Food Frequency Questionnaire and 7-Day Food Record

[d]Spearman correlation coefficients between energy-adjusted intakes reported on the Food Frequency Questionnaire and 7-Day Food Record

Table 2

Proportion and individual agreement in reporting intake of high sources of N-nitroso food groups by 7-Day Food Record and Food Frequency Questionnaire in the N-Nitroso Validation Study

Food	% Reporting Food Group Eaten		*P* Value[a]
	7-Day Food Record	Food Frequency Questionnaire	
Beef	83	84	0.25
Sausage	51	70	0.05
Bacon	47	66	0.005
Wine	33	57	<0.0001
Hot Dog	23	56	<0.0001
Ham	38	41	0.88
Beer	20	20	0.89

[a] X^2, McNemar statistic