Null Results in Brief

Null Association between Frequency of Cured Meat Consumption and Methylvaline and Ethylvaline Hemoglobin Adduct Levels: The *N*-Nitroso Brain Cancer Hypothesis[1]

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Introduction

Numerous animal experiments have shown definitively that methylnitrosoureas, and particularly ethylnitrosoureas, are potent neurocarcinogens (1). Furthermore, experimental studies have demonstrated that ingestion of nitrites and alkylureas results in the formation of alkylnitrosoureas in the stomach, which can be delivered transplacentally to the fetus leading to malignant brain tumor formation in offspring of exposed dams (2). In the human diet, cured meats such as hot dogs, bacon, packaged lunch meats, cured ham, and sausage are commonly treated with nitrites to inhibit botulism and to enhance the visual appeal of the meats (3). Accordingly, nonexperimental human studies have been conducted to evaluate the potential association between cured meat consumption by mothers during pregnancy and the subsequent elevated risk of brain tumors in their children (4). Although far from conclusive, the majority of these epidemiological studies have provided suggestive evidence in favor of the relation (5).

The hypothesized alkylnitrosourea-brain cancer causal pathway can be summarized as follows: ingestion of nitrites facilitates the formation of alkylnitrosoureas in the acidic stomach environment. Alkylnitrosoureas, which are highly reactive and do not require metabolic activation, decompose into diazonium ions. Diazonium ions react to form promutagenic O^6-alkylguanine DNA adducts. O^6-alklyguanine adducts are efficiently repaired by the O^6-alkylguanine alkyltransferase DNA repair protein. This repair mechanism, however, is subject to a high degree of phenotypic variation, and those with the MER− phenotype, in contrast to the MER+ phenotype, have poor capacity for repair (6). Accumulation and persistence of O^6-alkylguanine adducts can lead to tumor formation (7). In this pilot study, we sought to provide evidence consistent with this hypothesis, that the formation of methylnitrosurea and ethylnitrosurea is higher from foods treated with nitrites, *i.e.*, cured meats, by evaluating differences in methylation and ethylation of NH_2-terminal valine in Hb.[3] Hb adducts have been used effectively as biomarkers of exposure to carcinogens and other alkylating agents (8, 9). Advantages of hemoglobin adducts include the relatively long lifetime of the red cell (~120 days), the ease of obtaining adequate quantities of material, and the lack of any known repair processes. We hypothesized that persons who consume cured meats would, in a dose-response fashion, have substantially higher mean blood levels of NH_2-terminal Hb-alkylvaline biomarkers for alkylnitrosourea formation, MV and EV, than persons who do not consume cured meats.

Materials and Methods

After approval from the Human Subjects Review Board, we recruited volunteers from students, staff and faculty at the University of Minnesota to provide a brief dietary questionnaire and two blood samples drawn 1 month apart. Subjects were required to be nonsmoking adults who were not taking vitamin C or vitamin E supplements, which may inhibit formation of alkylnitrosoureas (Ref. 10; subjects were not excluded if they took a daily multiple vitamin). We attempted to include an even distribution of non-meat eaters, non-cured meat eaters, and cured meat eaters (*i.e.*, the dietary groups). Of the 114 subjects recruited, 40 reported being vegetarians who consumed no meat; 39 reported eating meat products in their diet, but usually not cured meats; and 35 reported inclusion of cured meat products in their regular diet. We determined mean EV and MV levels using a modified Edman degradation procedure originally developed by Tornqvist (11). Reaction of globin with pentafluorophenyl isothiocyanate results in formation of a pentafluorophenylthiohydantoin (PFPTH) derivative that can be quantified by gas chromatography-negative ion chemical ionization-mass spectrometry. The derivatives, MeVal-PFPTH and EtVal-PFPTH, result from methylation and ethylation, respectively of NH_2-terminal valine. Using this method, we determined mean levels of EV and MV over the two blood draws and averaged their values. We used general linear modeling-least squares regression analysis to evaluate whether mean values of EV and MV differed across dietary groups or by quartiles of weekly cured meat consumption while controlling for potentially confounding variables. We had 80% power to detect a mean EV difference of 1.38 pmol/g and mean MV difference of 95 pmol/g between each dietary group, assuming a two-sided type I error level of 5%.

Results

We found detectable levels of EV in 103 (90%) of our subjects and detectable levels of MV in all subjects. Both mean EV and

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[3] The abbreviations used are: Hb, hemoglobin; MV, methylvaline; EV, ethylvaline.

Table 1 Ethylvaline and methylvaline globin levels by cured meat consumption

Study subjects	Weekly servings of cured meat mean (range)	EV blood level, pmol/g mean (SE)	Adjusted[a] EV blood level, pmol/g mean (SE)	MV blood level, pmol/g mean (SE)	Adjusted[a] MV blood level, pmol/g mean (SE)
Grouped by dietary status					
Vegetarians, $n = 40$	0.04 (0–1.0)	2.6 (0.33)	2.5 (0.36)	558 (21.8)	570 (23.9)
Noncured meat eaters, $n = 39$	2.0 (0–9.5)	3.2 (0.34)	3.2 (0.35)	637 (22.1)	647 (22.9)
Cured meat eaters, $n = 35$	5.2 (1.0–10.5)	2.9 (0.37)	3.0 (0.38)	585 (23.4)	593 (24.0)
P[b]	<0.001	0.43	0.33	0.039	0.042
Grouped by quartiles of cured meat consumption					
Lowest 25%, $n = 41$	0 (0)	2.6 (0.33)	2.5 (0.35)	565 (22.1)	575 (24.0)
Mid-low 25%, $n = 27$	1.3 (0.5–2.0)	3.6 (0.42)	3.6 (0.42)	615 (27.2)	626 (28.0)
Mid-high 25%, $n = 27$	3.3 (2.5–4.5)	2.6 (0.41)	2.8 (0.41)	612 (27.2)	625 (27.9)
Highest 25%, $n = 19$	7.2 (5.0–10.5)	3.1 (0.48)	3.2 (0.48)	595 (32.4)	600 (32.5)
P[b]	<0.001	0.25	0.19	0.43	0.42

[a] Adjusted for sex, age, and average weekly consumption of foods high in antioxidants.
[b] Testing the null hypothesis that all means are equal.

mean MV, as predicted, were lower in the vegetarian dietary group than in either of the meat-eating groups; these differences, however, were small and could well be attributed to random variability. We did not observe a consistent dose gradient of increasing EV or MV levels with increasing cured-meat consumption (Table 1).

Discussion

Several study limitations should be considered when evaluating these essentially null findings: (*a*) although our dietary groups differed in their frequency of consumption of cured meat products (Table 1), we had few heavy cured meat eaters. We cannot rule out the possibility that heavier consumption of cured meats would result in greater differentiation of EV and MV between vegetarians and meat eaters; (*b*) we recruited a volunteer study group of working adults who do not smoke, and we make no assumption that they represent a fair sample of the population at large. Our true target population of interest is pregnant women, but feasibility issues precluded recruitment of such a sample for this study; (*c*) the quantity of added nitrites in cured meats has been decreasing in recent years (3), and it may be that nitrite levels are not currently high enough to produce a clear differentiation of EV and MV between dietary groups; and (*d*) although some evidence exists to show that alkylnitrosoureas can be produced endogenously from food sources (10), studies are needed to assess the degree to which endogenous alkylureas are present in foods or in the stomach to react with nitrites.

Etiologies for childhood brain tumor occurrence, unfortunately, are largely unknown. The compelling evidence from animal experiments showing neurocarcinogenicity from alkylnitrosourea exposure and the success in such studies of inducing malignant brain tumors in offspring through transplacental exposure, makes this an appealing model to study in humans (5). The epidemiological research showing increased brain cancer risk among children whose mothers consumed cured meats during pregnancy, albeit often with internal inconsistencies in results, provide further justification for continuing research into this hypothesized causal pathway. Although our findings in this pilot study failed to show clear differences in a surrogate marker for alkylnitrosourea formation among persons who eat cured meats compared with those who do not eat meat, we encourage continued research efforts to elucidate whether this relation as hypothesized can be shown to be a likely contributor to neurocarcinogenesis in children.

References

1. Tomatis, L. Prenatal carcinogenesis. *In:* T. Kakundaga, T. Sugimura, L. Tomatis, and H. Yamasaki. Cell Diff Genes and Cancer, Sci. Pub. No. 92, pp. 121–132. Lyon: IARC, 1988.

2. Ivankovic, S. Teratogenic and carcinogenic effects of some chemicals during prenatal life in rats, Syrian golden hamsters, and minipigs. J. Natl. Cancer Inst. Monogr., *51:* 103–115, 1979.

3. Lijinsky, W. *N*-Nitroso compounds in the diet. Mutat. Res., *443:* 129–138, 1999.

4. Preston-Martin, S., Pogoda, J. M., Mueller, B. A., Holly, E. A., Lijinsky, W., and Davis, R. L. Maternal consumption of cured meats and vitamins in relation to pediatric brain tumors. Cancer Epidemiol. Biomark. Prev., *5:* 599–605, 1996.

5. Gurney, J. G., Smith, M. A., Olshan, A. F., Hecht, S. S., and Kasum, C. M. Clues to the etiology of childhood brain cancer: *N*-nitroso compounds, polyomaviruses, and other factors of interest. Cancer Investig., *19:* 630–640, 2001.

6. Silber, J. R., Blank, A., Bobola, M. S., Mueller, B. A., Kolstoe, D. D., Ojemann, G. A., and Berger, M. S. Lack of the DNA repair protein O^6-methylguanine-DNA methyltransferase in histologically normal brain adjacent to primary human brain tumors. Proc. Natl. Acad. Sci. USA, *93:* 6941–6946, 1996.

7. Bignami, M., O'Driscoll, M., Aquilina, G., and Karran, P. Unmasking a killer: DNA O^6-methylguanine and the cytotoxicity of methylating agents. Mutat. Res., *462:* 71–82, 2000.

8. Ehrenberg, L., and Osterman-Golkar, S. Alkylation of macromolecules for detecting mutagenic agents. Teratog. Carcinog. Mutagen., *1:* 105–127, 1976.

9. Skipper, P. L., and Tannenbaum, S. R. Protein adducts in the molecular dosimetry of chemical carcinogens. Carcinogenesis (Lond.), *11:* 507–518, 1990.

10. Sen, N. P., Seaman, S. W., Baddoo, P. A., Burgess, C., and Weber, D. Formation of *N*-nitroso-*N*-methylurea in various samples of smoked/dried fish, fish sauce, seafoods, and ethnic fermented/pickled vegetables following incubation with nitrite under acidic conditions. J. Agric. Food Chem., *49:* 2096–2103, 2001.

11. Tornqvist, M. Epoxide adducts to N-terminal valine of hemoglobin. Methods Enzymol., *231:* 650–657, 1994.

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