Nitrate Levels in Community Drinking Waters and Risk of IDDM

An ecological analysis

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OBJECTIVE— To investigate whether higher IDDM incidence rates occurred in areas with high nitrate levels in their potable water supplies.

RESEARCH DESIGN AND METHODS— Incidence rates for the 63 counties in Colorado were calculated using the Colorado IDDM Registry of children diagnosed <18 yr of age between 1978 and 1988 (n = 1280). A weighted average of the nitrate levels from each water district within each county was calculated using data collected by the Colorado Department of Health between 1984 and 1988.

RESULTS— The r_S between nitrate levels and IDDM incidence was 0.26 (P = 0.03). After controlling for differences in ethnicity, counties with water nitrate levels in the third tertile (0.77–8.2 mg/L) had a significantly increased risk of IDDM compared with those in the first tertile (0.0–0.084 mg/L) (r_p = 0.29, P = 0.02).

CONCLUSIONS— This ecological analysis suggests that low-level nitrate exposure through drinking water may play a role in the etiology of IDDM, perhaps as a promoter through the generation of free radicals.

The large variations in IDDM incidence within and across populations (1) and the observed temporal trend of increasing incidence of IDDM in several European countries (2–8) suggest the contribution of an environmental factor that varies geographically and over time in the etiology of IDDM. Among factors that have been implicated in the etiology of IDDM, the role of nitrate exposure deserves further attention in light of a recent epidemiological investigation from Sweden that demonstrated that diabetic children consumed a greater amount of foods containing nitrates, nitrites, or nitrosamines before diagnosis than age-matched nondiabetic children (9). In addition, the concentrations of nitrate in groundwater in Europe have been increasing over the past 50 yr (10–12), attributable in part to increased use of nitrate-based fertilizers. Because as much as 70% of total nitrate intake is waterborne in areas of high water nitrate levels (13), waterborne nitrate may be an important, heretofore unmeasured, exposure in IDDM etiology.

The aim of this study was to determine if higher IDDM incidence rates existed in geographic areas that have higher nitrate levels in their potable water supplies.

RESEARCH DESIGN AND METHODS

IDDM population

The Colorado IDDM Registry is an ongoing registry designed to ascertain all new cases of IDDM <18 yr of age who were residents of the state of Colorado at the time of diagnosis. Eligible subjects had to have been placed on insulin within 2 wk of diagnosis and did not have diabetes secondary to other causes. A description of the registry's methods has been reported previously (14). A total of 1376 individuals were diagnosed with diabetes in Colorado between 1978 and 1988. Residence at diabetes diagnosis was obtained from physician report, medical records, or questionnaire response from the IDDM case. The 96 cases with unknown county of residence at diabetes diagnosis were excluded from these analyses. The study population had a mean age at diagnosis of 9.9 ± 4.4 yr and was 53% male.

The county IDDM incidence rates were calculated using the entire Colo-

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IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; r_S, SPEARMAN'S CORRELATION COEFFICIENT; r_p, PARTIAL CORRELATION COEFFICIENT; PI, PREDICTED INCIDENCE; PY, PERSON-YEARS.

rado IDDM Registry population of cases diagnosed between 1978 and 1988. The midpoint (1983) census population estimates of the 63 counties in Colorado were the denominators in the calculation of incidence rates. Because non-Hispanic whites have higher IDDM rates than Hispanics and blacks (1,15), the ethnic distribution of the county may explain some of the variability in county incidence rates. Therefore, the percentage of each county that was non-Hispanic white was calculated to control for different ethnic distributions by county.

Measurement of nitrate

The Water Quality Control Division of the Colorado Department of Health monitors public water supplies in Colorado and generates nitrate levels data by water district within each county and by sample date. Surface water systems are tested annually, and well water systems are tested once every 3 years. Private water supplies are not routinely tested by the Colorado Department of Health. The automated cadmium reduction method (Environmental Protection Agency Code 109) was used for nitrate analysis. The water nitrate data available for analysis were those collected between 1984 and 1988.

A representative nitrate index was computed for each county by calculating a weighted average of the mean nitrate values from each water district within the county. The weighting was accomplished by using the proportion of the population served by the water district to the population served by all water districts in the county. Weighted mean nitrate levels ranged from 0.0 to 8.2 mg/L. The county water nitrate data are available from the author (J.N.K.).

Statistical analyses

Because IDDM incidence follows a Poisson distribution rather than a normal distribution, the square root of the rate was used to stabilize the variance (16). Because this transformation did not entirely normalize the variable, an r_S, which is a measure of association between the ranks of two variables, was appropriate because the statistic is relatively insensitive to outliers and does not require normally distributed populations. To control for ethnicity, multiple linear regression models were fitted to the data using the SAS statistical package (SAS, Cary, NC) with the square root of the county IDDM incidence rates as the dependent variable, and weighted water nitrate levels and ethnic proportion of the county population as independent variables. Inspection of the residuals of this analysis revealed that the nonnormal distribution of the dependent variable did not compromise the model, and r_P's were calculated. Because of the skewness of the county ethnic proportion variable, it was divided into tertiles (1, 0.14–0.78; 2, 0.79–0.92; 3, 0.93–0.98) for analysis in the regression model. Because it is not known whether or not the relation between water nitrate and IDDM is linear, the nitrate variable also was analyzed in tertiles (1, 0.0–0.084 mg/L; 2, 0.085–0.76 mg/L; 3, 0.77–8.2 mg/L) to remove the assumption of a linear effect.

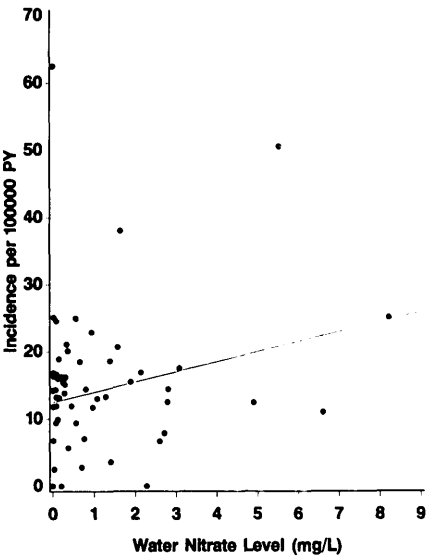

Figure 1—*County incidence of IDDM by weighted mean nitrate level (mg/L) in public water supplies. Colorado IDDM Registry, 1978–1988. (●), One county.*

RESULTS — County incidence rates of IDDM were plotted against the weighted mean nitrate level of the county's public drinking water systems (Fig. 1). The simple correlation (r_S) between county IDDM incidence and water nitrate level was 0.26 ($P = 0.03$) overall; and $r_S = 0.27$ ($P = 0.03$) for non-Hispanic whites only. After controlling for the proportion of non-Hispanic whites in the county population, water nitrate level was positively correlated with county IDDM incidence ($r_p = 0.23$, $P = 0.07$, model $R^2 = 0.10$). Water nitrate levels were divided into tertiles for additional analyses. While controlling for ethnicity, rates of IDDM were increased in counties where nitrate levels in the public water systems were in the 3rd tertile ($r_p = 0.29$, $P = 0.02$, PI = 15/100,000 PY), compared with those in the 1st tertile (PI = 7/100,000 PY, model $R^2 = 0.14$). Incidence rates in counties in the 2nd nitrate tertile were marginally elevated ($r_p = 0.23$, $P = 0.08$, PI = 13/100,000 PY) over those of the 1st tertile.

CONCLUSIONS — Contaminated water represents one route of nitrate exposure in man. Vegetables that have absorbed excess nitrate and preserved meats and fish are two other routes of exposure. The exposure estimates of this study are crude because we have no way of estimating dietary intakes of nitrate through vegetables and cured meats. Also, to determine with certainty whether an individual had been served by a public water supply or by a private well was not possible in this ecological study. A number of rural areas in Colorado are served primarily by private wells, which may have very high nitrate levels (17). In Colorado, although 16–100% of each county was served by public water supplies, this was not related to IDDM rates nor water nitrate level (data not shown).

To obtain more stable estimates of county IDDM incidence, rates were

calculated using cases ascertained between 1978 and 1988 rather than between 1984 and 1988, which is when the nitrate data were collected. Water testing done before 1984 suggests that nitrate levels in Colorado have not changed during the 1980s (G. Bodnar, BS, Public Health Engineer, Colorado Department of Health Water Quality Control Division, unpublished observations).

The role that nitrates play in diabetes etiology may be related to the endogenous formation of diabetogenic nitrosamines. Under pH conditions similar to those of the gastrointestinal tract (18), nitrate can be reduced to nitrite which, in the presence of secondary amines, forms nitrosamines. Previous studies have shown that toxic doses of nitrosamines or *N*-nitroso compounds can cause diabetes (19–21) via the generation of free radicals that damage the insulin-producing β-cells of the pancreas. The effect of low-level nitrate exposure on diabetes risk is less clear. In Sweden, diabetic children consumed a greater amount of foods containing nitrates, nitrites, or nitrosamines before diagnosis than nondiabetic children (9). However, without water nitrate data, this study may have underestimated the total nitrate exposure and excluded a source of potential variability. Additionally, circumstantial evidence suggests a connection between IDDM in males and consumption of nitrosamine-rich smoked mutton by their parents at the time of conception (22).

Currently, it is unclear whether nitrosamines act as initiators or promoters of the diabetogenic process. Our ecological study was designed to evaluate nitrate exposure as a promoter rather than an initiator of IDDM, because we were examining exposure to water nitrate around the time and place of IDDM diagnosis rather than in utero or during infancy. The relatively small proportion of the variance in IDDM rates that is explained by nitrate exposure can be attributed to several issues: incomplete assessment of nitrate exposure, inappropriate timing of the exposure measurement, and/or lack of data on the individual. Future studies are needed to confirm this finding in other IDDM populations, to incorporate both food- and water-borne nitrates in the exposure assessment, and to determine whether the timing of the exposure is important to its diabetogenic effect.

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