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Project Summary

Assessment of Cadmium Exposure and Toxicity Risk in an American Vegetarian Population

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It has been postulated that nonvegetarians may be exposed to less cadmium than vegetarians because of the cadmium-poor meat in their diet. This study attempts to test this possibility by measuring the cadmium exposure and accumulation in a population subgroup that includes many vegetarians. The conclusions are: 1. no statistical difference in cadmium exposure rate is demonstrated between the nonvegetarians and lacto-ovo-vegetarians of this study; 2. the cadmium exposure rate of the small group of pure vegetarians studied is in the direction anticipated (higher than nonvegetarians) but is not large enough to achieve statistical significance; 3. cadmium accumulation in the critical organ (kidney) of subjects coming to autopsy is well below the range associated with renal injury; and 4. quantitative methods for measuring total urine protein are not as satisfactory for assessing tubular proteinuria as those developed to measure the small proteins that normally appear in the glomerular filtrate.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Only 3% of the cadmium used in the United States is recycled. The remainder is dispersed into the biosphere where it may contaminate our drinking water and enter the food chain. Estimates based on autopsy data and environmental analysis have led to the fear that lifetime exposure and accumulation may be nearing a critical toxic level in the general population. Schroeder's animal experiments and several autopsy series have suggested a relation between unrecognized cadmium accumulation and hypertension. Up to 20% of the total body burden in smokers may be related to absorption from tobacco-born cadmium. Analysis of foods from cadmium-contaminated regions shows variation in cadmium content. Cereals and other foods of plant origin contain higher concentrations than most foods of animal origin. Regardless of whether cadmium enters the body via the gastrointestinal tract from food and water or the respiratory tract via smoke or dust, the physiologic biochemical pathways result in its eventual binding to metallothionein. This 5000 dalton protein has the greatest binding affinity for cadmium of any protein studied thus far and easily carries the cadmium with it through the glomerular basement membrane into the renal tubular filtrate. The normal tubular function for conservation of amino acids and proteins results in the removal of cadmium-metallothionein complex from the filtrate into the tubular mucosa. The cadmium remains sequestered in the renal cortex for the rest of the life of the individual. When a suf-

ficient concentration has accumulated, renal tubular dysfunction with cadmiumuria, beta-2-microglobuluria, and Fanconi syndrome become apparent. Additional accumulation leads to destruction of the renal cortex. The scarred contracted end stage kidney is indistinguishable from that of glomerulonephritis, pylonephritis, or hypertensive renal disease.

It has been postulated that non-vegetarians may be exposed to less cadmium than vegetarians because of the cadmium-poor meat in their diet. The food animal is exposed to the same water and plant sources as the human and the cadmium accumulates in his kidneys by similar physiologic mechanisms as in the human. As a consequence the skeletal muscle is depleted of its cadmium. Steak is cadmium-poor; sweet breads, especially kidneys and liver, are not. Food derived from skeletal muscle will dilute the cadmium obtained from the cadmium-accumulating food plants.

This study attempts to test this possibility by measuring the cadmium exposure and accumulation in a population subgroup that includes many vegetarians. The study group is drawn from Southern California Seventh-Day Adventists (SDA) already enrolled in the ongoing Adventist Health Study. Seventh-Day Adventists are a small Protestant denomination with about three million members worldwide. Approximately 100,000 live in California. Church standards require that members abstain from the use of tobacco and alcoholic beverages. Previous studies have shown that over 98% of church members conform to these standards. The church also recommends other practices affecting life style that are not used as criteria for membership. A vegetarian diet is urged as the ideal. The use of unclean meats as biblically defined (pork, shellfish, etc.) is proscribed. Use of caffeine-contained beverages (coffee, tea, colas) and strong condiments (such as black pepper) is discouraged. Whole grains, nuts, vegetables and fruits as major dietary elements are recommended. A food industry specializing in protein products derived from plant sources has developed. Although these dietary practices have been taught for over 100 years, members vary widely in actual practice. A very few are pure vegetarians or vegans (PV). About half are lacto-ovo-vegetarians (LV), who use eggs and dairy products but abstain from meat, poultry and fish. The remaining non-vegetarians (NV) use clean meats and fish in their diet. About 17% drink one or more cups of coffee per day. Epidemiologic studies among this group began a quarter of a century ago in the content of unhealthful effects of tobacco usage. These led to the prospective Adventist Health Study begun in the 1970's. Approximately half of the California Adventist population are enrolled. They completed life style questionnaires and respond periodically to health status inquiries. Hospital record and death certificate information supplements health data obtained directly from the participants.

The public concern for identifying and minimizing environmental health risks mandates a monitoring of exposure rates. Following index population groups that have higher than average exposure to cadmium may be useful in providing warning of general population health risk.

Participants

Live Subjects

From the computerized records of the Adventist Health Study, 50 pairs consisting of one SDA lacto-ovo-vegetarian and one SDA non-vegetarian were generated. The members of each pair were matched by age, sex, marital status, education, menopausal status, and occupational group. Since cadmium accumulates over the life span of the individual at a biological half life of approximately 20 years, these subjects were selected from the 40- to 60-year-old age group. Another matching was attempted to develop a similiar pairing with 50 pure vegetarians. Because these vegans are such a small fraction of those enrolled in the Adventist Health Study, the age requirements were relaxed to increase the possibility of recruiting a complete group. These subjects were identified only by a transformed identification number. The ID numbers of the subjects that matched were passed back to a computer routine that mailed letters explaining the study to subjects. Each subject was randomly assigned to one of the two dieticians for the rest of the study. The dietician was given a name, address, and phone number. Any further information the dietician received about the subject came directly from the subject. If a subject declined to participate, none of our staff learned anything further about the subject, even whether or not he was part of the Adventist Health Study.

Five to seven days after the introductory letter was mailed, the dietician called the participant. She briefly explained the study, answered any questions, and made an appointment for the first home visit. If the subject agreed to participate, a letter confirming the appointment and an introductory manual briefly explaining specimen collection was mailed. At the first interview, a written consent was obtained and a detailed explanation of the collection procedure made both verbally and in the form of an extensive instruction manual.

When a subject declined to participate, a computer routine was used in an attempt to replace him. If he was a vegetarian subject the computer searched the files for another match. If this was unsuccessful, the dietician assigned to contact the matching subject was instructed to cancel the appointment for home visit with the matching subject, and expressed our regrets. If another match was found, and home visits with the original subject had not been scheduled, they were postponed until a few days after the new subject had received the introductory letter.

Once the first home visit had been completed, all subjects were carried through the completion of sample collection, regardless of withdrawals of matched subjects. The first visit was conducted by a research nutritionist. Any questions at this point were answered. Written consent was obtained from the study subject. Then the subject was asked to recall food intake in the previous 24 hours, using models and following a prod question sheet. The purpose of the unannounced recall was to determine if the subjects changed their diets while completing the three-day diary. Seventh-Day Adventists in particular may have a number of ideas about an appropriate diet which they may not be following exactly. The knowledge that their diet is being analyzed may cause them to consciously or unconsciously change their eating.

A health history (exclusion criteria) questionnaire was administered by the nutritionist. This questionnaire included inquiries about a number of characteristics that were the basis for further exclusions. These included osteomas, major changes in diet in the last two years, and use of a number of drugs, including cancer chemotherapeutic agents and lipid lowering agents. The use of estro-

gens was recorded, but not used as grounds for exclusion.

The subject was instructed in the collection of urine and feces. A 2.5-liter container and a 1-liter widemouthed plastic jar in an airline bag were provided for 24-hour urine collected on the second day of feces collection. This excluded the first voiding of that day and included the first voiding of the following day. The container provided contained a preservative (HC1). The fecal samples included every stool during three days. Plastic bags were provided to hang in the toilet, or a port-a-potty made available if so desired. After defecation, the bag was removed, deposited in another plastic bag and the outer bag closed, and placed in a freezer chest packed with dry ice. This outer bag was labeled with the subject's identification number, the date, whether contaminated with urine, and a sequential number to identify the bowel movement. Subjects were instructed to urinate before defecating to minimize urinary contamination of the fecal sample. The plastic bag was hung in the toilet in such a way that there was some space in front of the bag for males to urinate. In case of females, a small amount of urine might fall into the bag. There also was space beind the bag to drop paper into the toilet. A small checklist was used to identify any stools with urinary contamination. Employed subjects were provided with another freezer chest at their place of employment if they felt it likely they would defecate at work. This was labeled "Biological Materials for Scientific Research—Do Not Disturb or Remove." Our staff contacted the employer to deliver and pick up the chest.

All subjects were asked to collect food samples. All foods and beverages (except water) consumed were weighed on a gram scale. During the three days of fecal collection, the subject was asked to place about 2 ounces of food or beverage other than water, in a small plastic container labeled with the names of the food. Dressings, sauces, etc., added at the table were collected separately, as these may not be mixed homogeneously on the plate with the foods with which they are eaten. A number of small containers, each containing 2 ounces of a different food, could be placed in a larger container labeled for the data and the meal. The food samples were refrigerated, but not frozen. Vitamin pills and other dietary supplements were collected in exact duplication of the amount used by the subject, and a record of the nutritional supplement's composition obtained by the dietician.

Each subject was given forms to record his complete dietary intake during the three days of fecal collection. He was instructed to record all food and beverages consumed, and to save recipes of homemade foods.

The subject was given a day within the following next week to begin his dietary record and sample collection. Each collection period included one weekend day; that is, it was Thursday, Friday, and Saturday, or Sunday, Monday, and Tuesday.

The 24-hour recall was coded by the dietician for the dietary analysis the same day if possible.

On the second day of the collection period, the same dietician phoned the subject to review the procedures and answer any questions. This allowed difficulties to be handled early. Specific questions were asked to ascertain comprehension and compliance.

On the workday following the sample collection (that is, a Monday or a Wednesday), the dietician retrieved the freezer chest containing the fecal and urine samples, another containing the food samples, and when used, a third containing samples from the subject's place of employment.

The dietician reviewed the three-day diary with the subject and edited the diary as necessary.

The subject's height, weight, triceps skinfold and blood pressure were measured.

The dietician checked each food cup for sample adequacy and completeness and reviewed the urine and fecal samples collected for completeness.

The same day the dietary diary was coded whenever possible.

The dietary interviewer made an appointment for a venipuncturist to draw blood, and explain the 12-hour fast. This appointment was made as soon as possible after the period of food collection, within no more than seven days. It was not made during the food collection period, or on the day following, to prevent the fast from interrupting the subject's usual eating habits.

The venipuncturist visited the subject's home, usually early in the morning to draw the blood samples for the biochemical profiling. Those subjects living within easy driving distance from the medical center were scheduled to have the blood work drawn in the outpatient venipuncture station.

Autopsy Subjects

Kidney, liver, pancreas and hair samples collected by cooperating pathologists in Southern California Seventh-Day Adventist hospitals were obtained from subjects coming to autopsy. Life style and health data were obtained from hospital charts, next-of-kin, and from the Adventist Health Study data base.

Conclusions

1. No statistical difference in cadmium exposure rate is demonstrated between the non-vegetarians and lacto-ovo-vegetarians of this study.
2. The cadmium exposure rate of the small group of pure vegetarians studied is in the direction anticipated (higher than non-vegetarians), but is not large enough to achieve statistical significance.
3. Cadmium accumulation in the critial organ (kidney) of subjects coming to autopsy is well below the range associated with renal injury.
4. Quantitative methods for measuring total urine protein are not as satisfactory for assessing tubular proteinuria as those developed to measure the small proteins that normally appear in the glomerula filtrate.

Discussion

Anticipated differences in cadmium exposure rates between vegetarians and non-vegetarians are dependent upon the replacement of cadmium-rich calories from cadmium-accumulating foods by cadmium-poor calories. For this difference to be apparent, the food of the vegetarian must contain more cadmium than that of the non-vegetarian. The ideal situation for demonstrating the difference is in the case of a single major food source for each group. This was the case for the Japanese eating rice grown in cadmium-contaminated water. But for the situation where there are a variety of foods, the difference may be masked. The non-vegetarian may be getting higher than background exposure by including liver, kidney and shellfish in this diet. The vegetarian who obtains the major portion of his food from the national food distribution system averages his exposure by the mixing of foods grown in cadmium-poor districts with those of contaminated areas. Another difficulty in demonstrating differences is in the classification of the subjects. In the strictest sense there are no pure vege-

tarians unless they grow all the food they eat. Processed foods usually include milk or egg solids and other non-vegetable additives and anyone depending upon the prepared food industry is getting more than he realizes. Self assignment to pure vegetarian, lacto-ovo-vegetarian or non-vegetarian groups may represent the person's ideal for his dietary practice, rather than his invariant practice. Of those pure vegetarians in the Adventist Health Study we contacted, most used some dairy products occasionally. Even the non-vegetarian group may be subject to cultural influence from its Adventist background to eat less meat than the general population does.

The lack of difference between the non-vegetarian group and the lacto-ovo-vegetarian group in this study is probably due to several factors. In spite of attempts to control confounding factors by matching subjects by age, sex, occupational group, etc., there still remain many differences (genetic, geographic origins, past changes in lifestyle) to mask diet-induced differences. Both groups are subject to environmental contamination from tobacco smoke and other erratic sources. Both replace a portion of the higher cadmium content plant foods in their diet by cadmium-poor foods—dairy products for the vegetarians and skeletal muscle meat cuts for the non-vegetarians. It is unfortunate that we could not recruit a larger group of pure vegetarians to reduce the dietary overlap.

The subjects in the autopsy study are not easily categorized into well-defined dietary groups. A few were lifelong lacto-ovo-vegetarians but many became Adventists later in life and had made significant life style and dietary changes. Several had been heavy cigarette smokers. Thus, the probable rate of exposure had varied significantly at different times of their lives. Even so, the total renal cortex accumulation remained below the risk threshold of 200 micrograms cadmium per gram of wet tissue.

The concentration of cadmium in flour used in making meat analogues is in the range reported by other studies. The highest value was from a sample obtained from a partially open flour bin in a market where it may have been contaminated by the environment.

Urine cadmium concentration was at the low end of the working curve of the instrument. Its measurement was facilitated by the concentration achieved by the extraction step.

Urine protein methods are tuned to measure albumin. In the typical clinical setting, the physician is most interested in proteinuria as an indicator of glomerular dysfunction. Since the major protein appearing in the urine as a consequence of glomerula injury is albumin, such methods meet the clinical need. They vary widely in their response to other proteins. The smaller proteins normally pass through the glomerular basement membrane, but are removed form the glomerular filtrate by the tubular epithelium. In the setting of tubular dysfunction or injury these small proteins replace albumin as the constituents of greatest clinical interest, but most methods grossly underestimate or fail to detect them. In our study, this methodological shortcoming is evidenced by the lack of correlation between beta-2-microglobulin excretion and total protein excretion. Tubular proteinuria must be looked for specifically by urine protein electrophoresis or by specific assay of one or more of the small protein species.

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***Norman Kowal** is the EPA Project Officer (see below).*
The complete report, entitled "Assessment of Cadmium Exposure and Toxicity Risk in an American Vegetarian Population," (Order No. PB 85-211 340/AS; Cost: $10.00, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
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