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Chronic Low-Level Mercury Exposure, BDNF Polymorphism, and Associations with Self-Reported Symptoms and Mood

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Recent reports have described neurobehavioral impairments in human subjects carrying a V66M polymorphism in the gene encoding brain-derived neurotrophic factor (BDNF). Inasmuch as ventral nervous system (CNS) deficits associated with this BDNF polymorphism are similar to those observed among subjects with chronic exposure to elemental mercury (Hg°), we examined the potential effect of this BDNF polymorphism on symptoms and mood in an established cohort of dental practitioners with chronic low-level Hg° exposure. Self-reported symptoms and mood were obtained by computerized questionnaire from 193 male dentists (DTs) and 230 female dental assistants (DAs). Spot urine samples were analyzed for mercury concentrations to evaluate recent exposure. Detailed work histories were obtained to calculate chronic indices of Hg° exposure. Buccal cell samples were obtained to identify the V66M polymorphism of BDNF. Scores for 11 current and 12 recent and chronic symptom groups, along with six mood factors, were evaluated with respect to recent and chronic Hg° exposure and BDNF polymorphism. Multiple regression analysis controlled for age, race, socioeconomic status, tobacco and alcohol use, self-reported health problems, and medications. Separate evaluations were conducted for DTs and DAs. Twenty-three associations between recent or chronic Hg° exposure and BDNF status and self-reported symptoms were observed with $p < 0.10$. All but three were in the expected direction (symptom scores increasing with Hg° exposure or BDNF polymorphism), and all but six were among DAs. All eight correlations between chronic exposure indices and recent and chronic symptoms among DAs were in the expected direction. All seven associations between BDNF and symptoms were in the expected direction and split between DTs and DAs. All three associations with mood factors were among DAs and in the expected direction. These results indicate that among DAs very low levels of occupational Hg° exposure are associated with increased symptoms. The BDNF polymorphism is also associated with increased symptom and mood scores. Notably, Hg° and BDNF polymorphism were additive with respect to their associations with the same symptom group.

Key Words: **mercury; brain-derived neurotrophic factor; BDNF; polymorphism; mood; symptoms; neurobehavioral.**

Brain-derived neurotrophic factor (BDNF) is a protein that regulates neuronal growth and differentiation in the peripheral and central nervous system (Burkhalter *et al*., 2003). It is hypothesized that a nucleotide polymorphism substitution of valine (val) to methionine (met) in the BDNF gene at codon 66 (val66met) can interfere with processing and secretion of the BDNF protein. The substitution may be a single (val-met) or a double (met-met) substitution. A number of recent human studies support this hypothesis. One study demonstrated significant differences in performance on an episodic memory test based on single BDNF substitutions (Egan *et al*., 2003). Two separate research groups have reported significant differences in hippocampal activation and deactivation (using functional magnetic resonance imaging) during the performance of memory tasks between subjects with single substitutions in BDNF and controls (Egan *et al*., 2003; Hariri *et al*., 2003; Marx, 2003). One group found that the interaction between BDNF val66me genotype and hippicampal response during encoding accounted for 25% of the variation seen on their test of episodic memory (Hariri *et al*., 2003). Other human health-effect reports associate variants in the BDNF gene with obsessive-compulsive disorders (Hall *et al*., 2003) and anorexia nervosa (Ribases *et al*., 2003). Serum BDNF levels are both associated with depression and modified by the use of anti-depressant medications (Shimizu *et al*., 2003). Long-term elemental mercury (Hg°) exposure has also been associated with similar CNS effects, particularly memory loss, depression, and anxiety (Clarkson, 1989, 2003).

Self-reported symptoms and mood have been sensitive indicators of exposure to a number of environmental neurotoxins, supplementing more objective behavioral performance tests and direct nerve conduction measures. While organic mercury, e.g., CH_3Hg^+, associated with consumption of fish and other sources, has captured much attention, exposure to elemental mercury through amalgam dental fillings is a prevalent and distinct source of exposure. Dentists and other dental personnel incur potential

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occupational exposure to elemental mercury (Hg°) from placements of mercury-containing amalgam in addition to that from their own personal dental fillings.

Initially reports on the health effects of elemental mercury were associated with high industrial exposures that could produce potentially severe and clinically recognizable neurological effects. These effects included salivation, loss of appetite, emotional lability, loss of mental capacity, and loss of motor function such as imbalance and tremor (Albers *et al*., 1988; Langworth *et al*., 1992; Pranjic *et al*., 2003; Piikivi *et al*., 1984; Piikivi and Hänninen, 1989). However, with further study, pre-clinical effects within these domains have been observed at lower, and even very low, levels of exposure (Clarkson *et al*., 2003; Langworth *et al*., 1997; Ngim *et al*., 1992; Ritchie *et al*., 2002; Shapiro *et al*., 1982; Soleo *et al*., 1990). Our own studies have shown significant neurobehavioral effects associated with elemental mercury exposures in the range of those experienced by the general population with mercury amalgam dental fillings (Bittner *et al*., 1998; Echeverria *et al*., 1995, 1998). These include reduced visual memory, reduced motor speed, and reduced hand steadiness. We have also seen increased symptoms and mood changes.

This article presents measures of self-reported symptoms and mood and their observed associations with current urinary mercury exposure levels and a cumulative mercury exposure index among dental practitioners. Additionally, the effect of the BDNF polymorphism on these associations and the possible interaction with mercury exposure was evaluated.

MATERIALS AND METHODS

The study population. In 1998, all licensed dentists in Washington State (n = 3,750) were mailed a packet that included a letter of introduction, an informed consent, a short questionnaire, and a urine collection kit with instructions. A total of 2,675 urine samples were returned. The short screening questionnaire was used to determine subject eligibility for additional studies. Criteria for inclusion into the ongoing study included (1) participation in an uninterrupted full-time dental practice for 5 consecutive years immediately prior to enrollment; (2) absence of health conditions that could alter urinary mercury levels including, but not limited to, kidney disease (e.g., lithiasis, pyelonephritis, orthostatic proteinuria), endocrine disorders, and cancer; and (3) no history of chelation therapy. Eligibility was further restricted to male dentists because the very small number of female respondents was insufficient for statistical modeling. A total of 1,488 male dentists met the study criteria.

The mean urinary mercury level among all dentists responding to this general survey was 2.5 μg/L (range = 0–67). The mean among eligible participants was 2.32 μg/L (SD = 1.49). We randomly recruited qualified dentists across exposure strata based on intensity of exposure to elemental mercury as determined by the screening urinary mercury level.

To evaluate the effects of dental mercury on women, we recruited female dental assistants from the practices of participating dentists. This method allowed relatively easy access to dental assistants, and had the added benefit of providing a study group pseudo-stratified on mercury exposure. Based on the assumption that intensity of exposure is strongly associated with office practices and parameters, we expected that the recruited dental assistants would have a range of mercury exposure similar to the range of participating dentists.

Between 1998 and 2001 we recruited 193 male dentists and 230 female dental assistants for evaluation of the effects of elemental mercury on the nervous system.

Data collection. Dentist and dental assistant participants were scheduled for evaluation at a central location. Participants first signed an informed consent, took a breath alcohol test, and provided a urine sample (~50 ml). Each participant completed a computerized questionnaire (Neuroquest) and a computerized behavioral test battery called the Behavioral Evaluation for Epidemiologic Studies (BEES) Test Battery (Echeverria *et al*., 2002). In addition, various paper-and-pencil tests and stand-alone neurological tests were also administered.

Neuroquest collects information on demographics, personal and dietary habits, medical and pregnancy histories, work histories, a symptoms checklist (45 symptoms), and a computerized version of the Profile of Mood States (McNair *et al*., 1971) to measure current mood.

Personal habit information included a detailed smoking history and histories of alcohol and caffeine use. Dietary habit information included current consumption of fish, shellfish, and vitamin and mineral supplements on a frequency per week basis.

Medical conditions are grouped into categories including (1) physical injury, (2) major operations, (3) digestive problems, (4) circulation problems, (5) sensory problems, (6) kidney problems, (7) endocrine problems, (8) immune problems, (9) brain-related problems, and (10) emotional problems. Yes/no information was collected on the presence of any condition, the presence of specific or 'other' conditions, and the current use of medications for any of these conditions within each category.

Data on current medication use was obtained through a paper-and-pencil questionnaire that was later computerized and coded into categories. These categories included (1) blood pressure control and anti-cholesterol drugs, (2) anti-depressants, (3) allergy drugs, (4) birth control/menopause (female assistants only), (5) acid reflux drugs, (6) thyroid drugs, (7) epilepsy drugs, (8) opoids and percadone, (9) topical drugs, (10) other drugs, and (11) supplements. Within each category, summary data including use (yes/no), number of drugs used, and the sum of each drug by its frequency of use were calculated.

Symptoms were assessed in conjunction with an extensive neurological battery of computerized and paper-based behavioral tests, hand steadiness, postural sway, and nerve conduction velocities that are reported elsewhere. Blood samples were collected to control for organic mercury and possible lead exposures, and buccal swab samples were collected for DNA analyses.

Urinary Hg analyses. Analysis of total mercury was performed using continuous flow, cold vapor spectrofluorometry, as previously described (Pingree *et al*., 2001). Urinary creatine concentrations were measured using a standard colorimetric procedure (Sigma #555-A). Urinary mercury levels were calculated as both μg/L of urine and μg/gm creatinine.

Genomic assays. Brain-derived neurotrophic factor (BDNF) genotyping was performed at the Functional Genomics Laboratory of the Center for Ecogenetics and Environmental Health at the University of Washington. A 5′-Nuclease TaqMan Detection System–based assay was developed to discriminate the G196A alleles in exon 2 of the BDNF gene. Primers and dual-labeled allele-specific probes were designed through Assays-by-Design Service-SNP Genotyping by Applied Biosystems Inc. (Foster City, CA). The polymerase chain reaction (PCR) primers are 5′-gCCCAAggCAggTTCAAgAg – 3′(sense) and 5′-AACTTTCTggTCCTCATCCAACAg – 3′ (antisense). Each TaqMan MGB probes consisted of an oligonucleotide labeled both with a particular 5′ reporter dye and a 3′ nonfluorescent quencher. The probes for this assay are 5′-VIC-ACTTTCgAACAC**g**TgATAg-MGBNFQ-3′ and 5′-6 FAM-CTTTCgAACAC**A**TgATAg-MGBNFQ-3′. Amplification was performed by initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s and annealing at 60°C. End-point analysis was performed on an ABI 7700 Sequence Detection System (Applied Biosystems Inc., Foster City, CA) to determine the genotypes. Appropriate positive controls consisting of DNA aliquots representing wild-type/wild-type, wild-type/mutant, mutant/mutant genotypes (characterized by DNA-sequencing), and a negative control (no DNA) were included in each assay performed. In addition, 10% of the identified alleles were randomly re-analyzed and compared to previous analyses for quality control of this genotyping assay.

The Mood questionnaire. Measures of mood were obtained using a computerized version of the well-established and validated Profile of Mood States

(POMS) questionnaire (McNair *et al*., 1971). The POMS has 65 questions and provides six mood factor scales. These include Tension-Anxiety (9 questions, score range 0–32), Depression-Dejection (15 questions, score range 0–60), Anger-Hostility (12 questions, score range 0–48), Vigor-Activity (8 questions, score range 0–32), Fatigue-Inertia (7 questions, score range 0–28), and Confusion-Bewilderment (7 questions, score range 0–24). These scores are derived from oblique factors and are thus somewhat correlated with one another. The Vigor-Activity score is negatively correlated with all the other scores. An overall score can be calculated by adding the first five scores and subtracting the Vigor-Activity score.

The symptoms questionnaire. We obtained symptom measures through a computerized symptom checklist of our own derivation. Symptoms were included on this checklist based on previous research and previously established symptom questionnaires. These included the Swedish Q16 symptom questionnaire for evaluating solvent toxicity (Lundberg *et al*., 1997; Smargiassi *et al*., 1998), its German extension (Ihrig *et al*., 2001), the Euroquest symptom questionnaire (Carter *et al*., 2002; Chouaniere *et al*., 1997; Karlson *et al*., 2000), and the symptoms questionnaire contained in the World Health Organization (WHO) Neurobehavioral Core Test Battery (Anger *et al*., 2000; World Health Organization, 1986). As several of these questionnaires were based on solvent toxicity, we were careful to add additional questions more closely associated with heavy metal toxicity.

Our approach to obtaining symptom information is unique in that it distinguishes between current (today) and recent (over the past 3 months) symptoms. In addition, recent symptoms are classified by their duration, allowing definition of chronic symptoms. All 45 symptoms included in our checklist are appropriate for assignment of recent and chronic scores. However, only 27 symptoms are appropriate for assignment of current scores, as they require more than one day to evaluate (e.g., ''write notes to remind myself''). We believe that distinguishing these various types of symptoms, an uncommon practice, is important for properly characterizing and understanding responses to toxic insults.

Current (today's) symptoms were scored using only their intensity, whereas recent symptoms were scored using the product of their intensity and frequency. Symptom intensity and frequency are measured using five-point scales. For intensity, the scale ranges from ''none'' to ''extreme'' (score range 0–4), while the frequency scale is from ''rarely'' to ''constantly'' (score range 1–5). Duration of chronic symptoms is also measured along a five-point scale ranging from ''less than one year'' to ''seven years plus.'' For these analyses, we have defined chronic symptoms as those lasting for at least one year. Thus, chronic symptom scores were set equal to their corresponding recent symptom score if they had duration of at least one year, and otherwise were set to zero.

To reduce the number of variables in these analyses, we created *a priori* symptom groups (see Table 1). This process resulted in 11 symptom groups for current (today) symptoms (each group encompassing one to four symptoms), and 12 symptom groups for ''recent and chronic'' symptoms (each group encompassing two to eight symptoms). Our symptom checklist and this *a priori* symptom grouping have been successfully used in a number of our previous studies. (Echeverria *et al*., 1995a, b; 1998).

The score for each symptom group is equal to the highest (maximum) individual symptom score within that group. This scoring scheme is based on the concept that different body systems might respond to toxic exposures through a variety of symptoms. As an alternative, we also evaluated group scores based on the sum of individual scores within each group. However, we found this approach did not accurately reflect symptom severity, because some subjects had ''globalized'' symptoms, and would accumulate scores much higher than those with more focused symptoms. Furthermore, we believed that it was neither appropriate to exclude the resulting "outlier" scores, nor to give them the analytical weight that the score demanded.

Statistical analyses. Cross-sectional analyses were conducted using SPSS. A data file was constructed which contained all symptom and mood scores, measures of exposure to elemental mercury (both current and chronic), the BDNF polymorphism score, and covariates. A natural log conversion of spot urinary mercury concentrations (in μg/L) was employed as a measure of each participant's current exposure. These conversions usually have a better linear

TABLE 1
NeuroQuest Symptom Questions (by a priori category)

Symptom category	Individual symptom	Today	Recent/Chronic
Memory	Trouble remembering things (1)		*
	Trouble remembering phone numbers		*
	Having to go back and check things		*
	Need to make notes to remember things		*
Confusion	Confusion (1)	*	*
	Difficulty concentrating	*	*
	Difficulty driving		*
Depression	Depression (1)	*	*
	Fatigue	*	*
	Tiring more easily than usual		*
	Sleeping more than usual		*
	Reduced sexual interest		*
	Unexplained weight loss		*
	Unexplained loss of appetite		*
	Trouble understanding newspapers		*
Anxiety	Anxiety	*	*
	Unexplained pains	*	*
	Unexplained perspiration	*	*
	Difficulty sleeping		*
Coordination	Lack of coordination	*	*
	Difficulty gripping objects		*
	Difficulty with screw top lids		*
	Trouble buttoning clothes		*
Mood	Unexplained irritability	*	*
	Unusual mood swings		*
	Being more excitable than usual		*
Headaches	Headache	*	*
	Dizziness	*	*
	Blurry vision	*	*
	Feeling lightheaded	*	*
Parasthesias	Trembling	*	*
	Numbness	*	*
	Feelings of pins and needles	*	*
Muscle	Muscle weakness	*	*
	Unexplained loss of muscle strength		*
Digestive	Cramps	*	*
	Nausea	*	*
	Diarrhea	*	*
	Indigestion	*	*
Skin	Excessively dry skin	*	*
	Skin rash or sores	*	*
Heart & Lung	Shortness of reath	*	*
	Persistent cough	*	*
	Chest pain or tightness	*	*
	Heart palpitations	*	*

Note.—45 Total Questions (18 Recent and Chronic Only); Scales: Five (5) levels of severity (''None'' to ''Extreme''); five (5) levels of frequency (''Rarely'' to ''Constantly''); five (5) levels of duration (''<1 year'' to ''7+years'').

association with toxic effects. Before conversion, the value one was added to each concentration so that a nondetectable urinary concentration of mercury would have the value of its natural log be zero, thus avoiding the analyses being overwhelmed by large differences in the value of the log for very small differences in urinary concentrations of less than 1 μg/L.

A chronic mercury exposure index was created for participants by summing the contribution of each of their reported dental-related jobs. Each job's contribution was calculated by taking the product of the average weekly number of mercury amalgam fillings or removals performed (as reported by the dentist or dental assistant), the duration of the job, and a weighting factor based on the time-period of the job [$1 \geq 1992, 1.5 = 1985–1992, 1.75 \geq 1972–1982, 2.0 \leq 1970$]. The weighting factor was included as it was clear from our previous analysis of urinary mercury levels in dental populations, and from comparisons with earlier studies, that dental exposure to mercury had dropped significantly over the last few decades. In fact, since 1998, the mean urinary mercury concentrations for dentists had begun to approach the concentrations observed among the general population. Finally, in the analyses, the square root of this index was employed to make its distribution closer to normal (reducing the extended right tail). This type of weighted exposure assessment has been employed in many occupational studies. In particular, we have employed it successfully in our previous dental studies (Echeverria *et al*., 1995a, 1998), in our work with styrene (Heyer *et al*, 1996; Luderer *et al*., 2004), and in our studies of perchloroethylene (Echeverria *et al*., 1995b).

The BDNF polymorphism were scored as zero for "wild type" or no substitution ("val-val"—observed in 68% of this population), one for a single substitution ("val-met"—28% of the study population), and two for a double substitution ("met-met"—4% of the study population).

Potential covariates evaluated in the analyses included demographic, dietary, medical history, and medication use factors. Simple correlations were run between all outcomes (mood and symptoms) and the risk factors (acute and chronic exposure and BDNF status) to determine which associations we should focus on (had a basic relationship). Simple correlations between these factors and the covariates were also run to evaluate which may be confounders. We were aware that symptoms could easily influence medication use and were cautious about the potential to over-control for covariates in our analyses. Stepwise regression analyses were performed to evaluate the effects of current and chronic exposure, BDNF, and covariate factors on the associations of primary concern. These evaluations resulted in a final base model for all analyses. This base model included all three risk factors (urinary mercury, chronic mercury index, and BDNF status), as well as age and race as independent variables. Additional covariates specific to each outcome were added when they were significant ($p < 0.05$) in the model. These additional covariates are all dichotomous variables regarding health history and included (1) have a physical impairment, (2) history of respiratory problems, (3) history of circulatory problems, (4) history of sensory problems, (5) history of endocrine problems, and (6) history of major operations.

Finally, dentists (males) and dental assistants (females) were analyzed separately. It is clear from the very structure of the study that these two groups are different in many ways, and that it would be difficult to control for these differences in the analyses. These differences include gender, age, education and training, social status and income, and the clear difference in their power relationship at work. These differences, along with the observed differences in their Hg° exposure and reporting of mood and symptoms clearly indicated that a separation of analyses was appropriate.

RESULTS

Initial analyses disclosed no significant confounding from dietary or medication factors. Those medication factors that were strongly related to symptoms were either not related or only weakly related to mercury exposure or BDNF status. They generally did not affect the direction and strength of the associations as measured by beta values. Two demographic factors, age and race, were important in a number of associations. Furthermore, several medical history questions were significant for some symptoms.

TABLE 2
Study Population Descriptives

Measures	Males ($N = 191$) Mean (SD)	Females ($N = 230$) Mean (SD)	Sig. Diff*
Age at evaluation	48.9 (7.8)	36.0 (9.1)	.000
Vocabulary score	10.6 (1.0)	8.2 (2.1)	.000
Highest academic education score	7.0 (1.0)	4.8 (1.0)	.000
INCOME in $1000's	168 (101)	51.0 (31.0)	.000
Nat. log urinary Hg (+ 1)	1.1 (.5)	0.88 (.55)	.000
Sqrt. weighted index for cumulative Hg	27.1 (20.6)	15.2 (12.3)	.000
Number of coffees per week	11.4 (14.5)	10.4 (9.3)	.414
Current alcohol drinks per week	4.2 (5.3)	1.9 (2.6)	.000
Current cigarettes per day	0.20 (1.2)	0.97 (3.4)	.001
Number of fish meals per week	1.7 (.7)	1.2 (.7)	.000
Vitamin supplements per week	2.5 (1.9)	2.0 (1.8)	.006
Group percentages			
Caucasian	96%	87%	.000
English as 1st language (%)	98%	97%	.387
Right-handed	97%	78%	.000
BDNF: Wild-type	69%	67%	.929
Single substitution	26%	29%	—
Full mutation	5%	4%	—
Use prescription drugs	29%	53%	.000
Use over-the-counter drugs	36%	39%	.534
Have a physical impairment	6%	9%	.240

*t-test for continuous variables, ANOVA for categorical variables.

Table 2 shows the characteristics of the 191 male dentists and 230 female dental assistants included in this study. While most participants in both groups were Caucasian, spoke English as their first language, and were right handed, the percentages for each of these were significantly higher for dentists than for dental assistants. As expected, dentists were older, scored higher on their vocabulary tests, had higher levels of education, and had substantially higher incomes. Coffee consumption was approximately equal between the two groups, but dentists consumed more alcohol (although moderate amounts) and fewer cigarettes. Concerning the three target risk factors, dentists had significantly higher spot urinary mercury concentrations and higher cumulative indices of exposure. Both groups had remarkably similar distributions of the BDNF polymorphism.

Table 3 shows the average symptom score and percentage of each gender group reporting symptoms by symptom category. The table also shows the average mood (POMS-Mood) scores. It is clear that dental assistants (females) reported substantially higher levels of symptoms than did dentists (males), indicating that dental assistants either suffer more symptoms or are more willing to report symptoms than their male (dentist) counterparts.

TABLE 3
Mean Symptom Score and Frequency of Reporting

	Males (n = 193)		Females (n = 230)	
Today's symptoms (Score & Count)	Mean (SD)	# Reported (%)	Mean (SD)	# Reported (%)
Confusion	.13 (.40)	22 (11%)	.48 (.69)	88 (38%)
Depression	.44 (.68)	67 (35%)	.73 (.85)	117 (51%)
Anxiety	.40 (.66)	62 (32%)	.64 (.89)	100 (43%)
Coordination	.05 (.30)	7 (4%)	.18 (.51)	34 (15%)
Moody	.08 (.34)	12 (6%)	.25 (.57)	44 (19%)
Headache	.13 (.38)	22 (11%)	.37 (.78)	54 (23%)
Parasthesias	.12 (.39)	21 (11%)	.31 (.70)	50 (22%)
Muscle	.04 (.26)	5 (3%)	.21 (.56)	38 (17%)
Stomach	.16 (.45)	26 (13%)	.44 (.80)	71 (31%)
Skin	.30 (.67)	42 (22%)	.97 (1.06)	129 (56%)
Lung	.20 (.57)	27 (14%)	.45 (.76)	73 (32%)
Recent symptoms (score & count)				
Memory	2.89 (3.57)	134 (69%)	4.25 (4.34)	198 (86%)
Confusion	.68 (1.60)	54 (28%)	1.91 (2.75)	129 (56%)
Depression	2.93 (3.30)	148 (77%)	6.15 (5.09)	203 (88%)
Anxiety	2.77 (2.86)	151 (78%)	5.29 (4.80)	209 (91%)
Coordination	.27 (1.36)	16 (8%)	1.80 (3.60)	102 (44%)
Moody	.88 (1.58)	68 (35%)	2.85 (3.53)	152 (66%)
Headache	1.30 (1.68)	122 (63%)	3.34 (3.54)	178 (77%)
Parasthesias	.74 (1.78)	51 (26%)	1.67 (3.20)	92 (40%)
Muscle	.29 (1.24)	20 (10%)	1.34 (2.54)	79 (34%)
Stomach	1.26 (1.70)	108 (56%)	3.28 (3.45)	179 (78%)
Skin	1.11 (2.21)	65 (34%)	3.78 (4.87)	149 (65%)
Lung	.78 (1.53)	61 (32%)	1.95 (2.97)	122 (53%)
Chronic symptoms (score & count)				
Memory	2.82 (3.61)	124 (64%)	3.90 (4.37)	175 (76%)
Confusion	.57 (1.37)	45 (23%)	1.63 (2.66)	108 (47%)
Depression	2.77 (3.31)	134 (69%)	5.40 (5.25)	179 (78%)
Anxiety	2.14 (2.72)	123 (64%)	4.32 (4.66)	173 (75%)
Coordination	.19 (1.04)	13 (7%)	1.34 (3.18)	77 (33%)
Moody	.68 (1.37)	55 (29%)	2.61 (3.46)	137 (60%)
Headache	1.07 (1.64)	97 (50%)	3.00 (3.67)	146 (63%)
Parasthesias	.67 (1.76)	43 (22%)	1.32 (2.89)	72 (31%)
Muscle	.23 (1.19)	13 (7%)	1.01 (2.30)	60 (26%)
Stomach	1.13 (1.70)	92 (48%)	3.11 (3.43)	170 (74%)
Skin	.99 (2.17)	56 (29%)	3.51 (4.92)	131 (57%)
Lung	.56 (1.32)	45 (23%)	1.61 (2.79)	99 (43%)
POMS – Mood Score				
Tension	5.27 (4.15)	—	7.97 (5.55)	—
Depression	3.13 (5.25)	—	5.24 (6.55)	—
Anger	3.78 (4.99)	—	5.75 (5.75)	—
Vigor	19.41 (4.91)	—	14.79 (5.81)	—
Fatigue	5.18 (4.53)	—	7.25 (5.27)	—
Confusion	3.05 (2.67)	—	4.66 (3.32)	—
Overall Total	1.00 (21.0)	—	16.09 (25.6)	—

The highest level of symptom reporting was for recent symptoms, with anxiety, depression, and memory loss reported by 91%, 88%, and 86%, respectively, of females. These were also the highest reported symptoms among dentists, with the percentage reporting these symptoms at 78%, 77%, and 69%, respectively.

Table 4 presents the regression analysis for all observed associations between the three risk factors (log urinary mercury = acute Hg° exposure; chronic mercury index = chronic Hg° exposure; BDNF polymorphism) and symptom scores or mood scales with an alpha probability of less than 10%. These results given (slope, standard error, beta, and significance levels) are for the specific risk factor within the full model. Of the 23 associations with symptoms observed at this significance level, 17 were among females (dental assistants), and 6 among males (dentists). In only three cases were the associations in the unexpected direction (italicized in Table 4), i.e., higher symptom

TABLE 4
Models for Symptom Scores and Mood with Current Exposure, Cumulative Index, and BDNF

	Males				Females			
Exposure type outcome	Slope	SE	Beta	Sig	Slope	SE	Beta	Sig
Log urinary mercury								
Today's symptoms								
Confusion					.182	.085	.144	.03
Anxiety					.192	.106	.119	.07
Recent symptoms								
Chest					*−.735*	*.348*	*−.135*	*.04*[a,c,g]
Chronic symptoms								
Chest					*−.853*	*.329*	*−.167*	*.01*[a,c,e]
Chronic mercury index								
Today's symptoms								
Anxiety	.006	.003	.177	.03				
Headaches	.003	.001	.164	.04[d]	.011	.005	.167	.03
Recent symptoms								
Coordination					.053	.021	.183	.01[c,d,h]
Memory					.052	.027	.148	.06[b]
Stomach					.037	.021	.134	.08[a]
Skin					.064	.030	.164	.04[b]
Chronic symptoms								
Coordination					.060	.019	.231	.002[d]
Depression					.058	.032	.136	.07[a,g]
Memory					.051	.027	.144	.06[b]
Skin					.060	.031	.153	.05[b]
Chest	*−.010*	*.005*	*−.157*	*.05*				
BDNF								
Today's symptoms								
Anxiety	.149	.083	.130	.08	.294	.106	.183	.006
Coordination	.064	.036	.123	.08[d]				
Stomach					.170	.097	.117	.08[c]
Recent symptoms								
Anxiety					1.503	.574	.173	.01
Chronic Symptoms								
Anxiety					1.237	.564	.146	.03
Chest	.359	.151	.166	.02				
POMS—mood								
Depression					1.563	.792	.131	.05[c]
Vigor					−1.381	.700	−.130	.05[a]
Overall (total)					5.748	3.032	.123	.06[a]

Note. Reported regression statistics are for each specific risk factor parameter. All models contain age and race as covariates.
Additional covariates in specific analyses are indicated by the following: [a]current smoking (cigs/day); [b]smoking packyears; [c]current alcohol (drinks per week); [d]physical impairment; [e]history of respiratory problem; [f]history of circulatory problem; [g]history of major operation; [h]History of sensory problem.

scores with low Hg° exposure or ''wild type'' BDNF status, and all three of these involved symptoms of the heart and lungs. All three associations with mood scales observed at this significance level were among dental assistants and in the expected direction.

There were only four significant associations between symptoms and acute exposure (log urinary mercury), all among dental assistants. Two associations were with today's symptoms (confusion and anxiety) and both of these were in the expected direction. The remaining two associations were with recent and chronic heart and lung symptoms, and they were in the unexpected direction.

There were 12 significant associations between symptoms and chronic exposure (as measured by the chronic exposure index), 3 among dentists and 9 among dental assistants. Among dentists, there were two associations in the expected direction for today's symptoms (anxiety and headache) and one in the unexpected direction for chronic chest symptoms. Among dental assistants, all these associations were in the expected direction. One was with today's symptoms (headache), four with recent symptoms (coordination, memory, digestive system, and skin), and four with chronic symptoms (coordination, depression, memory, and skin).

There were also seven significant associations between symptoms and BDNF status, all in the expected direction. Among dentists, there were three associations, two with today's symptoms (anxiety and coordination), and one with chronic heart and lung symptoms. Among dental assistants, there were four

TABLE 5
Regression Predicted Changes in Symptom Scores for Hypothetical Risk Factors

	Males			Females		
Exposure type outcome	Base score	Adjusted score	Difference	Base score	Adjusted score	Difference
Log urinary mercury						
Today's symptoms						
Confusion				.27	.56	+.29
Anxiety				.32	.63	+.31
Recent symptoms						
Heart and lung				*1.78*	*.59*	*−1.18*
Chronic symptoms						
Heart and lung				*2.04*	*.67*	*−1.37*
Chronic mercury index						
Today's symptoms						
Anxiety	.20	.50	+.30			
Headaches	.13	.28	+.15	.22	.77	+.55
Recent symptoms						
Coordination				.18	2.83	+2.65
Memory				3.12	5.72	+2.60
Digestive				2.41	4.26	+1.85
Skin				2.32	5.52	+3.20
Chronic symptoms						
Coordination				−.28	2.72	+3.00
Depression				2.56	5.46	+2.90
Memory				2.89	5.44	+2.55
Skin				2.27	5.27	+3.00
Heart and lung	*.53*	*.03*	*−.50*			
BDNF polymorphism						
Today's symptoms						
Anxiety	.20	.50	+.30	.32	.91	+.59
Coordination	.00	.13	+.13			
Digestive				.31	.65	+.34
Recent symptoms						
Anxiety				4.23	7.24	+3.01
Chronic symptoms						
Anxiety				3.28	5.75	+2.47
Heart and lung	.53	1.25	+.72			
Mood scales						
Depression				3.84	6.97	+3.13
Vigor				14.94	12.18	−2.76
Overall				12.32	23.82	11.50

associations, two with today's symptoms (anxiety and digestive system), one with recent anxiety symptoms, and one with chronic anxiety symptoms. In addition, among dental assistants, there were three associations between mood scales and BDNF observed at the alpha less than 10% level. These were for the depression, vigor, and overall scales, and all were in the expected direction (increased depression and overall scores, and decreased vigor with observed BDNF polymorphism).

It should be noted that interactions between mercury exposure and BDNF status with respect to their associations with symptom scores and mood scales were evaluated, but none was observed. It does appear, at least in these analyses, that these were multiplicatively independent factors. However, there were a number of cases where mercury exposure and BDNF status were additive with respect to their associations with the same symptom group. Among dentists, today's anxiety symptoms and chronic heart and lung symptoms were associated with both the chronic mercury index and BDNF status (although the association between chronic exposure and chronic chest symptoms was in the unexpected direction). Among dental assistants, today's anxiety was associated with both acute mercury exposure and BDNF status, whereas chronic memory loss was associated with both the chronic mercury index and BDNF status.

Table 5 is included to illustrate the actual projected magnitude of effects for the three risk factors on symptom scores based on theoretical regression analyses using selected baseline and exposed status. This is useful for understanding the real-world effects of dental mercury exposure. We use average observed age, dominant race (Caucasian), BDNF "wild-type" (no substitution), and no mercury exposure either currently or

historically as descriptors for our theoretical "baseline" population. Our theoretical exposed population is also based on average age and dominant race. However, their three risk factors are now BDNF set to double-substitution polymorphism (met-met), and both current and historical mercury exposures set to relatively high, but reasonable exposures (approximately one standard deviation above the observed average value for the dentist population). Thus, exposed status uses a urinary mercury concentration of 5 μg/L (the natural log of 5 is 1.6 or approximately one standard deviation above the average natural log of the urinary mercury concentration observed among dentists). The chronic exposure index for exposed status is 2,500 (with a square root of 50, or approximately one standard deviation above the average square root of the chronic index observed among dentists. Although Table 5 is based upon theoretical values, it does demonstrate that important increases in symptom severity and/or frequency can occur in these populations, especially if several risk factors are present at the same time.

DISCUSSION

The present results have several striking features that require discussion. First of these are the very high prevalence of some of the more important symptoms, especially anxiety, depression, and memory loss. Either very high or low prevalence can be a problem for studies that rely on dichotomous outcomes, as both create a lack of room for observing variation. However, our use of symptom scores rather than simple checklists has eliminated this problem. These results indicate that it is the intensity and frequency of symptoms that vary with risk factors, not simply their prevalence. The substantial variation between current and recent symptoms also suggests that this is a useful distinction.

The second striking feature in this study is the heavy predominance of observed associations among female subjects (dental assistants) as compared to males (dentists). While this may be associated with the higher prevalence of symptoms reported by females than males, it is more likely associated with females reporting a greater range of intensity and frequency than males, creating greater room for observing the association.

As stated earlier, dentists and dental assistants have many differences between them that may account for these observations. The distinct social roles men and women play in our society, and especially in the workplace, could be important factors explaining the observed differences. There may also be specific occupational factors involved. Dentists, unlike dental assistants, use vibrating tools (such as drills), which might be risk factors for some of these symptoms. The use of vibrating tools would not necessarily be associated with the level of mercury exposure given the increasing use of non-mercury fillings, and thus may mask otherwise observable associations. Nevertheless, our results are based on dose–response analyses conducted within each group separately. Thus, we would expect to see strong effects independent of the differences between these two groups.

Finally, the strength of this study is not in the high significance of the observed associations, many of which were borderline, but in the consistency of the observed change occurring in the expected direction. This is especially true for the associations between the chronic mercury index and symptoms where 12 of 13 associations (meeting the alpha less than 10% standard) were in the expected direction, and between BDNF polymorphism and symptoms, where all 8 associations were in the expected direction. In addition, all 3 associations meeting our criteria between BDNF polymorphism and mood scales also were in the expected direction.

Many of the symptom groups that have observed associations with exposure have previously been associated with toxic exposures in general and with Hg° exposure specifically. These include coordination, memory loss, anxiety, depression, and headaches. Those not commonly associated with Hg° exposure, and included for completeness and as control variables, include symptoms of the digestive system, skin, or heart and lung. There is no clear explanation for these associations. They may be random, or they may possibly be associated with other dental exposures that may coincide with mercury exposure, such as nitrous oxide. It is interesting that all three observations of associations in the unexpected direction are associated with symptoms of the heart and lung. We do not believe that these observations represent that Hg° acts as a protective factor for these symptoms.

CONCLUSIONS

This study presents significant evidence on the impact of low levels of Hg° exposure and BDNF status on symptoms and mood. Many researchers believe that when conducting studies with multiple outcomes it is necessary to use a corrected or stricter alpha cutoff level for considering results significant. We obviously do not concur in the case of this study. An adjusted alpha level can be appropriate when expecting that any one of many outcomes measured would be of importance with respect to the risk factors. However, as in this case, when we fully expected to see an array of results, we believe that it is far more important to assess the consistency of the direction of the association within the array of results than to worry about the individual alpha level of each result. If there were no true associations, we would expect to see chance observed associations randomly distributed across positive and negative directions. Under this assumption, the probability of seeing 20 out of 23 symptoms move in a particular direction is very low, approximately $p = .0002$. Similarly, the probability of seeing 12 of 13 symptoms associated with chronic exposures move in a particular direction is approximately $p = .0016$, and for 8 out of 8 symptoms associated with BDNF it is approximately $p = .0039$.

The public health significance of these finding is of particular concern. The levels of Hg° exposure among these dental personnel are not much greater than exposures to the general population through the dental amalgam in their fillings. Dentists are exposed to Hg° due to the placement of amalgam restorations containing 50% Hg° (Naleway *et al*., 1985). In our national sample of 6,925 dentists participating in the American Dental Association (ADA) Health Screen Program (1990–1996), 90% had urinary Hg levels under 6.0 μg/L. In Washington State, our sample of 2,196 dental professionals (1998–2000) had mean urinary Hg levels of 2.5 (0–67) μg/L. Re-testing in 2001 resulted in mean levels of 2.32 (1.49) μg/L.

Two independent estimates of urinary mercury levels among non-occupationally exposed populations have shown relatively low average levels of exposure associated with a wide range. Among a military cohort ($n = 1127$) a mean of 3.1 and range of 0–34 μg/L was observed (Kingman *et al*., 1998). Among employees of a New York City university health center ($n = 550$) a mean of 1.7 (range = 0.1–18) μg/g creatinine was observed (Factor-Litvak *et al*., 2003). The exposures observed in our study population overlap with these reported population values. Thus, they can be used to evaluate whether changes in behavior are associated with exposures at levels that are experienced by general (nonoccupational) populations.

Although the changes we observed in symptoms and mood represent small variations within the normal range of human behavior (pre-clinical), these results, if upheld by future research, suggest that elemental mercury has an impact on human symptoms at low levels. Furthermore, symptoms and mood can have a significant effect on quality of life. It is possible that elemental mercury may follow the history of lead, eventually being considered a neurotoxin at extremely low levels.

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