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A significant relationship between mercury exposure from dental amalgams and urinary porphyrins: a further assessment of the Casa Pia children's dental amalgam trial

David A. Geier · Thomas Carmody · Janet K. Kern · Paul G. King · Mark R. Geier

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Abstract Previous studies noted specific changes in urinary porphyrin excretion patterns associated with exposure to mercury (Hg) in animals and humans. In our study, urinary porphyrin concentrations were examined in normal children 8–18 years-old from a reanalysis of data provided from a randomized, prospective clinical trial that was designed to evaluate the potential health consequences of prolonged exposure to Hg from dental amalgam fillings (the parent study). Our analysis examined dose-dependent correlations between increasing Hg exposure from dental amalgams and urinary porphyrins utilizing statistical models with adjustments for the baseline level (i.e. study year 1) of the following variables: urinary Hg, each urinary porphyrin measure, gender, race, and the level of lead (Pb) in each subject's blood. Significant dose-dependent correlations between cumulative exposure to Hg from dental amalgams and urinary porphyrins associated with Hg body-burden (pentacarboxyporphyrin, precoproporphyrin, and coproporphyrin) were observed. Overall, 5–10% increases in Hg-associated porphyrins for subjects receiving an average number of dental amalgam fillings in comparison to subjects receiving only composite fillings were observed over the 8-year course of the study. In contrast, no significant correlations were observed between cumulative exposure to Hg from dental amalgams and urinary porphyrins not associated with Hg body-burden (uroporphyrin, heptacarboxyporphyrin, and hexacarboxyporphyrin). In conclusion, our study, in contrast to the no-effect results published from the parent study, further establishes the sensitivity and specificity of specific urinary porphyrins as a biomarker for low-level Hg body-burden, and also reveals that dental amalgams are a significant chronic contributor to Hg body-burden.

Keywords Body burden · Dental amalgam · Mercury · Porphyrin

D. A. Geier · T. Carmody
Institute of Chronic Illnesses, Inc., Silver Spring, MD, USA

J. K. Kern
Genetic Consultants of Dallas, Allen, TX, USA

J. K. Kern
University of Texas Southwestern Medical Center, Dallas, TX, USA

P. G. King
CoMeD, Inc., Silver Spring, MD, USA

M. R. Geier (✉)
ASD Centers, LLC, 14 Redgate Ct, Silver Spring, MD 20905, USA
e-mail: mgeier@comcast.net

Introduction

Porphyrins are formed as derivatives of the heme synthesis pathway, an essential biochemical pathway,

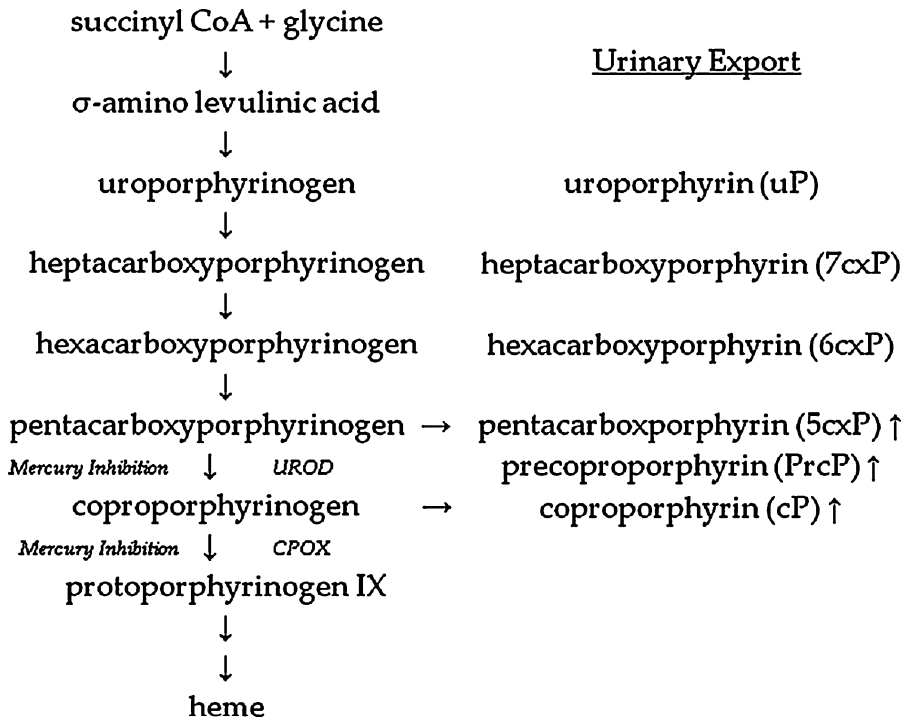

Fig. 1 A summary of the heme synthesis pathway and associated urinary porphyrins. Porphyrinogens appear in urine as porphyrin derivatives (right). Mercury can cause increased urinary 5cxP, PrcP, and cP by inhibiting uroporphyrinogen decarboxylase (UROD) and/or coproporphyrinogen oxidase (CPOX); urinary uP is not reported to alter with inhibition of these enzymatic steps

as shown in Fig. 1. Heme biosynthesis occurs in nearly all eukaryotic tissues. In humans and other mammals, porphyrins with eight, seven, six, five, and four carboxyl groups are commonly formed in excess of that required for heme biosynthesis and the excess amounts are excreted through the urine in a well-established pattern (Bowers et al. 1992; Woods et al. 1993).

Porphyrins can be utilized to afford a measure of xenobiotic exposure (Brewster 1988). In previous studies specific changes in urinary porphyrin excretion patterns (porphyrin profiles) associated with prolonged exposure of both animals and humans to mercury (Hg) and other metals were described (Woods and Fowler 1977, 1978; Fowler et al. 1987). The changes in urinary porphyrin excretion patterns were shown to involve both metal-induced inhibition of specific heme pathway enzymes in target tissues and metal-facilitated oxidation of reduced porphyrins that accumulate in tissue cells because of impaired porphyrin metabolism (Woods 1996). Changes in porphyrin excretion patterns are largely metal specific, correlate with metal concentrations in tissue cells, and occur prior to the onset of target tissue injury. Thus, investigators have found that urinary porphyrin profile measurements can be utilized as biomarkers of metal exposure and toxicity in human subjects (Woods 1996).

Previous studies have shown that urinary porphyrins can be particularly useful in measuring Hg exposure (Gonzalez-Ramirez et al. 1995; Geier and Geier 2006, 2007; Geier et al. 2009a, b; Kern et al. 2010a,b; Woods, 1996; Pingree et al. 2001). Urinary porphyrins are not a direct measure of Hg in the urine, but a measure of presence of Hg in the body (or Hg body-burden) by the level of disruption of the heme synthesis pathway that Hg causes. The presence of Hg inhibits specific enzymes that are necessary for the heme synthesis pathway to progress. This inhibition or interference results in a "backlog" and an increase urinary excretion of specific porphyrins. The level of increase in these "backlogged" metabolites measured in the urine correlates with the level of disruption of this pathway and indicates the extent of Hg tissue burden. There is a high degree of statistical correlation between renal Hg burden and the urinary excretion of specific porphyrins (Pingree et al. 2001). This correlation is consistently observed in animal models of Hg exposure; and the heme pathway is highly conserved across species (Gonzalez-Ramirez et al. 1995). Further, studies in humans have found

similar findings, i.e., that specific patterns of urinary porphyrins suggest the presence of Hg and the extent of the burden (Woods et al. 1993).

Specifically, Hg body-burden has been demonstrated to be associated with elevations in urinary coproporphyrin (cP), pentacarboxyporphyrin (5cxP), and by the expression of an atypical porphyrin–precoproporphyrin (PrcP) (also known as keto-iso-coproporphyrin) not found in the urine of unexposed controls. Several studies showed this characteristic pattern of porphyrinuria with Hg intoxication and also showed that elevated levels of Hg associated urinary porphyrins can be normalized with Hg-specific chelation therapy (Gonzalez-Ramirez et al. 1995; Geier and Geier 2006, 2007; Woods et al. 1993; Woods 1996; Pingree et al. 2001; Nataf et al. 2006). Woods (1996) noted that these distinct changes in urinary porphyrin concentrations were observed as early as 1–2 weeks after initiation of Hg exposure, and that they increased in a dose- and time-related fashion with the concentration of Hg in the kidney, a principal target organ of Hg compounds.

In addition, Hg-associated urinary porphyrin profiles not only correlate significantly with Hg body-burden, but also with specific neurobehavioral deficits associated with low-level Hg exposure. Echeverria et al. (1995), for example, examined the behavioral effects of low-level exposure to Hg vapor among dentists. These investigators observed that urinary porphyrins were as sensitive as urinary Hg levels for predicting adverse effects of Hg on cognitive and motor testing. Several studies have been completed recently that used urinary porphyrins to examine Hg toxicity in children, both in neurotypical children and in children with neurodevelopmental disorders (Geier and Geier 2006, 2007; Kern et al. 2010b; Nataf et al. 2006; Austin and Shandley, 2008; Young et al. 2010). Several of these studies examined the relationship between the severity of the neurological impairment and urinary porphyrins, and found significant positive correlations with Hg-associated urinary porphyrins and the severity of the neurological impairment (Nataf et al. 2006; Geier et al. 2009a, b; Kern et al. 2010a). In addition, one recent study examined two groups of neurotypical children with different levels of environmental Hg exposure and found that the level of Hg exposure was reflected in the Hg-associated urinary porphyrin levels (Kern et al. 2010c). Importantly, Woods et al. (2009) recently published a study supporting the utility of specific urinary porphyrins as biological indicators of subclinical Hg exposure in children.

In our study, urinary porphyrin concentrations were examined in children 8–18 years-old, with and without dental amalgam fillings, from a reanalysis of data collected from a completed clinical trial (the parent study) that was designed to evaluate the potential health consequences of prolonged exposure to Hg from dental amalgam fillings (DeRouen et al. 2006). Our study is designed to determine whether there was a significant dose-dependent correlation between increasing Hg exposure from dental amalgams and specific urinary porphyrins associated with Hg body-burden.

Materials and methods

The parent study protocol was approved by the institutional review boards at the University of Washington and the University of Lisbon. All parents or guardians gave written consent, and all children provided signed assent. Principal design and analytical issues involved in the parent study (DeRouen et al. 2002) as well as principal outcome measures (DeRouen et al. 2006) have been reported. Our study was undertaken by reanalyzing datasets provided to us by the investigators involved with the parent study.

Study population

The cohort of children we examined came from the parent study, which was the Casa Pia clinical trial on the health effects of dental amalgam fillings in children (DeRouen et al. 2006). As described previously (DeRouen et al. 2006), the children examined by us were residents of the Casa Pia school system in Lisbon, Portugal and were 8–12 years-old at the inception of the parent study. Eligibility requirements excluded children with preexisting neurological or developmental disabilities. Subjects were initially randomized to Hg amalgam (treatment) or composite resin (control) dental care groups. Children were evaluated at baseline and at seven subsequent annual intervals after the initial dental treatment. An extensive battery of neurobehavioral, neurological, renal function, urinary Hg, and urinary porphyrin assessments were used in each evaluation. In addition, detailed information was collected regarding the

Table 1 A summary of the baseline measurements for all subjects ($n = 462$) examined in the present study

Baseline measurements	
Mean age ± SD (years)	10.11 ± 0.9
Gender (% male)	57
Asian (%)	1
Black (%)	29
White (%)	70
Mean blood lead level ± SD (μg/dL)	4.63 ± 2.4
Urinary mercury level ± SD (μg/L)	1.48 ± 1.1
Uroporphyrin (μg/L)	8.56 ± 8.8
Heptacarboxyporphyrin (μg/L)	1.76 ± 2.8
Hexacarboxyporphyrin (μg/L)	0.43 ± 0.8
Pentacarboxyporphyrin (μg/L)	1.35 ± 3.1
Precoproporphyrin (μg/L)	3.56 ± 3.9
Coproporphyrin (μg/L)	34.84 ± 38.4

composition, number, size, and positioning of dental fillings in each child's mouth. Table 1 summarizes the baseline measurements recorded on the cohort of children ($n = 462$) examined by us from the parent study. In our analyses, we did not modify the original dataset provided to us from the parent study.

Urine sample collection procedures

As previously described (Woods et al. 2009), a urine sample (~50 ml) was collected from each child at baseline and at each subsequently scheduled annual visit to the University of Lisbon School of Dental Medicine for dental, neurological, and neurobehavioral evaluations. Porphyrins were quantitated in the remaining unacidified portion of the urine sample by high-performance liquid chromatography, as previously described (Bowers et al. 1992). In our study, the following measurements of urinary porphyrin concentrations (μg/L) were analyzed: uroporphyrin (uP), heptacarboxyporphyrin (7cxP), hexacarboxyporphyrin (6cxP), 5cxP, PrcP, and cP.

Statistical analyses

In all of our statistical analyses a two-tailed *P*-value of <0.05 was considered statistically significant. In our analysis, an individual's exposure was measured by counting the number of amalgam restorations of the buccal, distal, lingual, and occlusal surfaces (no amalgam restorations were recorded for medial or incisal surfaces). An exposure score for each individual was computed by applying scores of 1.0, 2.0, or 3.0 for small, medium, or large restorations, respectively, then adding these scores for each restoration of each tooth for each year. Other weighting schemes for large, medium, and small sizes were also considered (i.e., 1.0, 4.0, 9.0; 1.0, 8.0, 27; 1.0, 1.0, 1.0; and ln(1.0), ln(2.0), ln(3.0)). However, the weighting scheme that best correlated with urinary Hg levels (using only amalgam subjects who were 12 or older to avoid any issues with baby teeth) was 1.0, 2.0, 3.0. Thus, this weighting scheme was to create the yearly exposure scores used in all our subsequent analyses. In addition, adjustments were made for baby teeth which comprised 22% of restorations. Since baby teeth are smaller than adult teeth, the exposure for baby teeth was taken to be one half the exposure for adult teeth (i.e., with scaling factors of 0.5, 1.0, and 1.5 for small, medium, and large restorations). Further, as subject weights were not available, each individual's yearly exposure score was divided by the subjects estimated body-mass-index (BMI) based on the subject's age and gender. The estimated BMI scores for age and gender utilized in the present study were obtained from the Centers for Disease Control and Prevention's BMI 50th percentile clinical growth charts (http://www.cdc.gov/growthcharts/clinical_charts.htm#Summary). The yearly exposure scores for individuals were accumulated from year to year. Thus, for an individual a restoration contributed to exposure for the year it was placed and each subsequent year, unless a tooth had been lost in a given year, in which case its exposure contribution was set to zero for that year and all subsequent years. This procedure was applied to both baby teeth and adult teeth.

An individual's exposure score for each year was assumed to affect the outcome measure for the same year. The assumption that exposure in a year affected outcomes in the next year was also considered. However, the first assumption produced a better fitting model.

Both amalgam and composite groups were included in our analysis but all participants in the composite group had an amalgam exposure level of zero except for two subjects who received amalgam restorations in error. Since repeated measures were collected for each subject, a mixed-effects repeated-measures model was

used to estimate the relationship between exposure and outcome. Each model included terms for subject age as the repeated measurement factor, exposure, and the following covariates: gender, race, baseline level (i.e. study year 1) of urinary Hg, baseline measurements of each urinary porphyrin measures, and the baseline level of lead (Pb) in each subject's blood. Interaction terms were added if they contributed significantly to the model. Ordinarily, one would use study year as the repeated factor because it measures time from the beginning of the intervention. Since, the present analysis is not comparing intervention groups however, it is more reasonable to use age as the repeated factor. For all outcomes a log transformation was used to satisfy the normality requirement for the statistical procedure. Table 2 summaries mean Hg exposure from dental amalgams by the age of the study subjects.

Table 2 A summary of mean mercury exposure from dental amalgams[a] by age of study subjects[b]

Study subject's age (years)	Study subject's dental amalgam mean exposure
8	10.8
9	11.0
10	10.8
11	11.7
12	12.5
13	14.2
14	15.2
15	16.7
16	19.0
17	19.8
18	19.9

[a] Exposure was measured by counting the number of restorations using amalgam then an exposure score was computed by first giving scores of 1.0, 2.0, or 3.0 for small, medium, or large restorations, respectively, then adding these scores for each restoration of each tooth. Exposure for baby teeth was taken to be one half the exposure for adult teeth (i.e., 0.5, 1.0, 1.5 for small, medium, and large restorations). Each exposure score was divided by the subjects estimated BMI determined by the subject's age and gender. The exposure scores for each restoration done in a year were added together to form the score for that year and the scores were accumulated from year to year. If a tooth no longer existed at a given year the exposure was set to zero for that year and all subsequent years. This procedure was applied both to baby teeth and adult teeth

[b] Includes only subjects assigned to the dental amalgam group

Results

Table 3 summarizes the relationship between the cumulative exposure to Hg from dental amalgams and each urinary porphyrin outcome measurement in the model constructed. A significant exposure effect means that the outcome is significantly affected by the level of exposure after adjustment for covariates. A positive estimate means that higher levels of exposure are associated with higher levels of the outcome measured, while a negative estimate means that higher levels of exposure are associated with lower levels of the outcome measured.

Overall, there were statistically significant correlations between an individual's cumulative exposure to Hg from dental amalgams and their Hg-associated urinary porphyrins of 5cxP, PrcP, and cP. As shown in Table 4, Hg-associated urinary porphyrins increased by 5–10% over the 8-year course of the study among individuals exposed to an average number of amalgams among the study subjects from the amalgam group, in comparison to study subjects with no exposure to dental amalgams. By contrast, no significant correlations were observed between cumulative exposure to Hg from dental amalgams and the other urinary porphyrins of uP, 7cxP, and 6cxP.

Discussion

Our study found that the characteristic pattern of porphyrinuria associated with Hg body-burden, specifically, elevated 5cxP, PrcP, and cP were significantly correlated with dental amalgam exposure in a dose-dependent fashion. The higher level of Hg amalgam exposure resulted in higher levels of Hg-associated porphyrins. Further, consistent with previous studies that examined Hg exposure using porphyrins, the non-Hg-associated porphyrins showed no statistically significant relationship with amalgam exposure. The findings suggest a dose-dependent correlation between increasing Hg exposure from dental amalgams and the specific urinary porphyrins associated with Hg body-burden.

The findings from the present study are consistent with previous studies examining Hg exposure from dental amalgams and other biomarkers of Hg body-burden. For example, Dunn et al. (2008) examined US children over a 5-year period and found that the

Table 3 A summary of the relationship between cumulative exposure to mercury from dental amalgams[a] and outcome urinary porphyrin measurements

Outcome measurement (μg/L)	β-Coefficient[b]	Standard error	Degrees of freedom	*T* Statistic	*P*-value
Uroporphyrin (uP)	0.001	0.001	580	0.88	0.38
Heptacarboxyporphyrin (7cxP)	0.001	0.001	447	1.07	0.28
Hexacarboxyporphyrin (6cxP)	0.001	0.0005	430	1.80	0.073
Pentacarboxyporphyrin (5cxP)	0.0022	0.001	452	2.44	0.015
Precoproporphyrin (PrcP)	0.0024	0.001	427	2.11	0.036
Coproporphyrin (cP)	0.0037	0.002	414	2.47	0.014

[a] An individual study subject's exposure was measured by counting the number of restorations using amalgam then an exposure score was computed by first giving scores of 1.0, 2.0, or 3.0 for small, medium, or large restorations, respectively, then adding these scores for each restoration of each tooth. Exposure for baby teeth was taken to be one half the exposure for adult teeth (i.e., 0.5, 1.0, 1.5 for small, medium, and large restorations). Each exposure score was divided by the subjects estimated BMI determined by the subject's age and gender. The exposure scores for each restoration done in a year were added together to form the score for that year and the scores were accumulated from year to year. If a tooth no longer existed at a given year the exposure was set to zero for that year and all subsequent years. This procedure was applied both to baby teeth and adult teeth

[b] Each outcome estimate was adjusted for the baseline level (i.e. study year 1) of urinary mercury, each porphyrin measure, gender, race, and blood lead level

Table 4 Estimated mercury-associated urinary porphyrin levels by age and level of exposure

Age (years)	5cxP (zero exposure)	5cxP (ave. exposure)	PrcP (zero exposure)	PrcP (ave. exposure)	cP (zero exposure)	cP (ave. exposure)
8	2.60	2.65	4.18	4.28	26.44	27.39
9	2.58	2.64	4.49	4.60	28.30	29.40
10	2.55	2.61	4.69	4.82	29.85	31.12
11	2.51	2.58	4.77	4.91	31.03	32.48
12	2.47	2.54	4.72	4.87	31.79	33.41
13	2.42	2.50	4.54	4.70	32.10	33.91
14	2.36	2.44	4.25	4.42	31.94	33.92
15	2.29	2.38	3.88	4.05	31.32	33.46
16	2.22	2.32	3.44	3.60	30.26	32.54
17	2.14	2.25	2.97	3.13	28.82	31.21
18	2.06	2.17	2.50	2.64	27.05	29.52
19	1.98	2.09	2.04	2.17	25.03	27.52

5cxP pentacarboxyporphyrin, *PrcP* precoproporphyrin, *cP* coproporphyrin

number of amalgam restorations had a significant dose–response relationship with Hg urine levels. Likewise, Woods et al. (2007), in a longitudinal study in children, found urinary Hg concentrations were highly correlated with the number of amalgam fillings. Post-mortem studies also show this same dose-dependent central theme. Guzzi et al. (2006), for example, found that at autopsy, total Hg levels in all types of tissue were significantly higher in subjects with a greater number of amalgam surfaces (>12) compared with those with fewer amalgams (0–3). These authors also reported that the greater the number of amalgams, the greater the likelihood that Hg was found in the brain.

Several studies found this same dose-dependent occurrence when determining the rate of Hg absorption from amalgams. For example, Abraham et al. (1984) looked at Hg levels in blood and in mouth air before and after chewing in 47 persons with and 14 persons without dental amalgam restorations and found that blood Hg concentrations were positively correlated with the number and surface area of amalgam restorations. Olstad et al. (1987) also found a positive correlation between urine Hg concentration

and extent of amalgam restoration. Vimy and Lorscheider (1985) found that subjects with 12 or more occlusal amalgam surfaces were estimated to receive a daily Hg dose of 29 μg, as compared to subjects with four or fewer occlusal amalgam surfaces, for whom the dose was 8 μg.

The steps in the heme pathway most vulnerable to heavy metal inhibition are those in which uroporphyrin decarboxylase (UROD) and coproporphyrinogen oxidase (CPOX) are involved (Woods and Kardish 1983; Woods et al. 2005), as shown in Fig. 1. As described previously, the presence of Hg inhibits specific enzymes that are necessary for the heme synthesis pathway to progress. This inhibition or interference results in a "backlog" and an increase in urinary excretion of specific porphyrins. The level of increase in these "backlogged" metabolites measured in the urine correlates with the level of disruption of this pathway and indicates the extent of Hg tissue burden. Our study appears to further confirm this phenomenon, as each of the urinary porphyrins before the Hg-inhibited specific enzymes showed a step-wise decrease in statistical significance with exposure to dental amalgams (6cxP > 7cxP > uP).

Of particular interest to the investigation of metal-mediated changes in porphyrin metabolism are findings from studies in methylmercury (MeHg)-exposed rats demonstrating highly specific changes in the urinary porphyrin excretion pattern attributable primarily to Hg-induced alterations of heme biosynthesis in the kidney (Pingree et al. 2001). These changes are characterized by dose- and time-related increases in urinary concentrations of 5cxP and cP and also by the appearance of PrcP, an atypical porphyrin (molecular weight [mw] = 668) that elutes on high-performance liquid chromatography (HPLC) prior to coproporphyrin (mw = 655) (Woods et al. 1991). PrcP appears to be specific to Hg exposure, and its mechanistic etiology in this regard was previously described (Woods et al. 2005). Studies in human subjects with occupational exposure to elemental mercury (Hg^0) vapor demonstrated responses precisely comparable to those observed in MeHg-treated animals, attesting to the utility of porphyrin profiles as a specific measure of Hg exposure in either organic or elemental form. The sensitivity of porphyrin changes as a measure of Hg exposure and body burden was also described (Pingree et al. 2001). Notably, statistically significant increases in the concentrations of 5cxP, PrcP, and cP were demonstrated in animals with renal cortical Hg concentrations as low as 14 μg/g (Woods et al. 1991), nearly four-fold less than the renal Hg concentration at which significant changes in more conventional bioindicators of Hg exposure in humans were reported (Buchet et al. 1980; Rosenman et al. 1986).

Consistent with previous observations, the results of our study suggest that urinary porphyrin testing was able to detect the low-dose continuous exposure to Hg among individuals with dental amalgams. The relative 5–10% increases in the Hg-associated porphyrins observed over the 8 year course of the present study, suggest that dental amalgams for the average individual do not cause an acute high-dose exposure to Hg, but instead reflect a significant life-long continuous chronic low-dose exposure to Hg. As a result, our data suggests that the chronic low-dose exposure to Hg from dental amalgams continuously makes a significant contribution to Hg-associated urinary porphyrin levels, and hence to contributes to an ever-increasing body-burden of Hg. This type of phenomena is particularly evident when comparing the relative 5–10% increase in the Hg-associated porphyrins observed in our study with other studies linking elevated Hg-associated porphyrins with diagnosed Hg-related neurological disorders. For example, several recent studies revealed twofold to threefold significantly higher Hg-associated porphyrins among children diagnosed with neurodevelopmental disorders in comparison to neurotypical children (Geier and Geier 2006, 2007; Geier et al. 2009a, b; Nataf et al. 2006; Austin and Shandley 2008; Young et al. 2010; Kern et al. 2010a, b).

It is noteworthy that the results from our study are in sharp contrast to those published from the parent study (Woods et al. 2009). It was previously reported from the parent study that no significant differences between treatment groups (amalgam vs. composite) were found when comparing all subjects for any of the porphyrins of interest. The differences in results from the parent study and our study appear to be the result of the detailed statistical models we constructed to evaluate multiple co-variables and detailed analyses of exposure variables in the data, which helped to reveal significant dose-dependent relationship between Hg exposure from dental amalgams and Hg-associated urinary porphyrins.

Strengths/limitations

In considering our study, the design utilized in the parent study was strong, and its strength helped to reduce any potential limitations. The overall design of the parent study was constructed a priori to the actual examination of any study subjects, and the study subjects examined were randomly assigned to dental amalgam or composite groups at baseline. As a result, biases regarding potential reasons for exposure to a specific treatment or regarding specific types of evaluations undertaken on study subjects should not have adversely impacted the data analyzed. In addition, the sizes of both the amalgam and composite groups were of moderate size and not numerically weighted in a direction (i.e. there were several hundred study subjects in both the amalgam and composite groups), so that these factors helped reduce potential biases regarding the sample composition in the statistical modeling utilized in the our study.

After initial random assignment of study subjects to amalgam/composite groups, the study subjects then had detailed information collected regarding specific biological parameters at baseline. Subsequently, detailed information was collected regarding exposure to dental amalgams (i.e. size, number, location, etc.) and repeated measurements of specific biological parameters. As a result, the repeated measurements examined in the parent study were collected in a controlled fashion, so that potential limitations, such as changes in sample collection techniques or analysis over the course of multi-year study of study subjects, should have minimally impacted the data we examined.

Among the limitations of our study, the parent study included minimal information regarding past exposure to Hg or other sources of Hg exposure during the study among the study subjects. As a result, it is possible that these unaccounted for sources of Hg may have created confounding in the data examined, helping to reduce the significance of the findings observed in our study. Despite this potential, our study found significant correlations. In addition, our study has the limitation that only individuals who were healthy at initial presentation were allowed entry into the parent study. As a result, the biological effects observed in our study from dental amalgam exposure may reflect those specific to healthy individuals, and not necessarily the consequences of Hg exposure from dental amalgams in less-than-healthy individuals. Another potential limitation of our study is the length of follow-up. Study subjects were followed for only 8 years in the parent study. Hence, it was not possible to evaluate the potential long-term consequences of dental amalgam exposure over the course of decades to these individuals. Despite this fact, over the course of the 8 years of our study, Hg exposure from dental amalgams did significantly impact the biological parameters examined. In addition, a further potential limitation of our study was the moderate size of the sample examined. It is possible that with a larger sample size, the correlations we observed would be more robust, and hence, even less likely to be the result of chance. Also, with a larger sample size or with an increased capability to account for potential confounding in the data, the "backlogging" phenomenon created by Hg's inhibition of specific enzymes necessary for the heme synthesis pathway may have resulted in a statistically significant association between additional urinary porphyrins and exposure to dental amalgams.

Conclusion

Our study, in contrast to the published results of the parent study, found that the characteristic pattern of porphyria associated with Hg body-burden, specifically, elevated 5cxP, PrcP, and cP were significantly correlated with dental amalgam exposure in a dose-dependent fashion. The higher level of Hg amalgams exposure resulted in higher levels of Hg-associated porphyrins. Further, consistent with previous studies that examined Hg exposure using porphyrins, the non-Hg-associated porphyrins showed no statistically significant relationship with amalgam exposure. As a result, this study helps to further establish the utility of urinary porphyrins as a biomarker of low-level Hg body-burden.

In addition, the relative 5–10% increases in the Hg-associated porphyrins observed over the 8 year course of the present study, suggest that dental amalgams for the average individual do not cause a significant acute exposure to Hg, but instead represent a significant life-long source of chronic exposure to Hg, with a continuing impact on increasing Hg body-burden. It is of theoretical concern from

extrapolation of our results that over the course of a life-time of 70 years, the contribution to Hg body-burden from dental amalgams may eventually result in elevations in urinary porphyrins similar to those observed in individuals with diagnosed neurological conditions associated with Hg intoxication, and hence result in Hg body-burden levels associated with Hg toxicity. As a result, in any study of dental amalgam safety, a follow-up period of decades would be advisable in order to determine accurately the actual long-term pathological adverse effects amalgam may induce, even in initially healthy individuals.

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Conflict of interest None of the other authors has any conflicts of interest concerning the present study.

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