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Abstract. Dental amalgam is the major source of inorganic mercury (Hg) exposure in the general population. The objective of the present study was to obtain data on changes in Hg levels in blood, plasma, and urine following removal of all amalgam fillings during one dental session in 12 healthy subjects. The mean number of amalgam surfaces was 18 (range, 13 to 34). Frequent blood sampling and 24-hour urine collections were performed up to 115 days after amalgam removal, and in eight subjects additional samples of plasma and urine were collected up to three years after amalgam removal. A transient increase of Hg concentrations in blood and plasma was observed within 48 hours after amalgam removal. In plasma, the peak concentrations significantly exceeded the pre-removal plasma Hg levels by, on average, 32% (1.3 nmol/L; range, 0.1 to 4.2). No increase in the urinary Hg excretion rate was apparent after amalgam removal. An exponential decline of Hg was seen in all media. Sixty days after the amalgam removal, the Hg levels in blood, plasma, and urine had declined to approximately 60% of the pre-removal levels. In seven subjects, who were followed for up to three years, the half-lives of Hg in plasma and urine were calculated. In plasma, a bi-exponential model was applied, and the half-life was estimated at median 88 days (range, 21 to 121). The kinetics of Hg in urine (nmol/24 hrs) fit a mono-exponential model with a median half-life of 46 days (range, 35 to 67). It is concluded that the process of removing amalgam fillings can have a considerable impact on Hg levels in biological fluids. After removal, there was a considerable decline in the Hg levels of blood, plasma, and urine, which slowly approached those of subjects without any history of amalgam fillings.

Key words: human, blood, plasma, urine, dental amalgam, environmental medicine, kinetics.

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Introduction

During the last decade, increased attention has been paid to the possible toxic effects caused by low-level mercury (Hg) exposure from dental amalgam fillings (MFR, 1992; US Public Health Service, 1993). It is well-established that amalgam fillings release Hg and constitute the major source of non-occupational exposure to inorganic Hg in adults in the Western world. Estimates of the daily uptake of Hg from amalgam have most often been calculated from data based on measurements of Hg vapor concentrations in intra-oral air. The estimates fall within a wide range, from 2 to 20 µg/day (WHO, 1991; US Public Health Service, 1993). It is generally assumed that the major absorption route is inhalation of evaporated Hg. In a number of studies, significant correlations have been shown on a group basis between the Hg concentrations in body fluids and the amount of amalgam fillings (Olstad *et al.*, 1987; Åkesson *et al.*, 1991; Langworth *et al.*, 1991; Jokstad *et al.*, 1992; Sandborgh-Englund *et al.*, 1994; Schweinsberg, 1994). Although no relationship seems to exist between impaired health and the number of dental amalgam surfaces (Ahlqwist *et al.*, 1988, 1993), a considerable number of people have had their amalgam fillings replaced to get rid of symptoms allegedly due to Hg from amalgam fillings. From a risk assessment point of view, it is of interest to reveal the impact of extensive dental treatment, *i.e.*, amalgam removal, on the Hg levels in blood and plasma, and to relate this to the influence of the daily Hg uptake from amalgam fillings. Factors to be considered in this respect are the rates of absorption, distribution, and elimination, *i.e.*, the kinetics of Hg. Such information is important for understanding the toxicological effects of Hg in chronic, low-dose exposure, *e.g.*, from dental amalgam. The aim of the present study was to obtain information on the kinetic patterns of Hg in blood, plasma, and urine following extensive dental treatment. Data on microbial resistance, mercury concentrations in saliva and feces, and renal function are presented elsewhere (Edlund *et al.*, 1996; Sandborgh-Englund *et al.*, 1996; Björkman *et al.*, 1997).

Table 1. Details of participating subjects

Subject	Age[a]	Gender	Body Weight[b]	Amalgam[c]	Removal Time[d]
100	39	f	45	20	65
101	27	m	85	13	70
102	33	f	60	14	25
103	44	f	73	15	35
104	30	f	70	21	50
105	52	f	67	14	50
106	38	m	75	17	75
107	37	f	71	16	35
108	46	f	62	34	40
109	36	m	87	24	50
110	37	f	56	13	40
111	48	f	58	15	20
Median	38		69	16	45
Range	27–52		45–87	13–34	20–75

[a] Age in years.
[b] Body weight in kg.
[c] Number of amalgam-filled tooth surfaces (1–5 *per* filling).
[d] Duration of the dental procedure, *i.e.*, removal of amalgam fillings (minutes).

Materials and methods

Twelve healthy subjects with a mean age of 39 (range, 27 to 57) years and a mean of 18 (13 to 34) amalgam-filled tooth surfaces participated (Table 1). None of the subjects had been occupationally exposed to Hg, nor had they had any dental treatment for at least one year. To minimize the influence of exposure to methylmercury (MeHg), the participants agreed to exclude fish from their diet one month prior to and until two months after the day of amalgam removal. All subjects underwent a physical examination including blood chemistry (B-hemoglobin, B-leukocytes, and erythrocyte sedimentation rate) and urinary dipstick test (Ecur-4 test, Boehringer Mannheim, GmbH, Germany) prior to the experiment. All values were found to be within normal ranges.

The study was approved by the local Ethical Committee at Karolinska Institutet. All subjects gave informed consent to take part in the study and to have their amalgam fillings exchanged for other dental materials.

Experimental procedure

About a week (5 to 7 days) before the amalgam removal, four blood samples were collected from the subjects on two consecutive days at 8 a.m. and 1 p.m. All urine was collected for 2 x 24 hrs on the same days. During the experimental day, all amalgam restorations were removed by conventional dental procedures (*i.e.*, high-speed water-spray cutting and vacuum evacuator, but no rubber dam). The median duration of the dental procedure was 45 min (range, 20 to 75; Table 1). Local anesthetics were used in two cases (subject #103, Citanest®; subject #106, Xylocain®). The fillings were substituted by conventional temporary filling materials (IRM®, Pulpisol®, Cavit®, or Nobetec®). Blood sampling was regularly performed at 3, 7, 24, 31, 48, 72, and 96 hrs after the start of the dental procedure. Additional blood samples were taken in the morning at 7, 14, 21, 30, 60, 85, and 115 days after amalgam removal. Twenty-four-hour urine collections were generally performed during the day of the blood sampling. To obtain data on the long-term impact of amalgam removal on Hg in plasma and urine, we took samples from eight subjects (#102, 103, 105, and 107 to 111) from one to three years after the removal.

Blood samples were collected from the antecubital vein into metal-free, heparinized glass tubes (Vacutainer®, Becton and Dickinson, Europe, Meylan, Cedex-France). The samples for plasma analyses were centrifuged, usually within one hour, at 1000 g for 15 min, and the plasma was transferred by Pasteur pipettes into polypropylene tubes (Sarstedt®, Nümbrecht, Germany).

Urine was collected in 500-mL wide-necked, polyethylene bottles and stored at +4°C. After the bottles were shaken vigorously, the urine portions from each 24-hour period were pooled into a 2500-mL polyethylene bottle. Sub-samples of the 24-hour portions were transferred into polypropylene tubes (Sarstedt®, Nümbrecht, Germany). All samples were frozen within 24 hrs and kept at -18°C until analyzed. All bottles were acid-washed and rinsed with high-purity water so that Hg contamination would be avoided. Bottles and test tubes were checked to be free of Hg contamination. Creatinine in urine was determined with a modified Jaffé method (Bergman and Öhman, 1980).

Mercury determination

Samples collected within 4 months after amalgam removal.—Hg in blood, plasma, and urine was determined in acid-digested samples (Skare, 1972; Einarsson *et al.*, 1984) by a cold-vapor atomic absorption technique after pre-concentration on an amalgamation trap made of gold wire (Bergdahl *et al.*, 1995). The detection limit (blank + 3 SD) in 0.2-mL samples was 0.8 nmol/L. All samples were analyzed in duplicate. The coefficients of variation (CV) calculated from the duplicate determination were 4% in blood, 6% in plasma, and 7% in urine. External reference samples from an interlaboratory comparison program (Centre de Toxicologie du Québec, Canada) and one commercial reference sample of lyophilized whole blood (Seronorm, batches #010010 and 205052, Nycomed, Oslo, Norway) were used to check the accuracy. The external reference samples of blood and urine, with Hg concentrations in the interval of 23 to 78 nmol/L, reached, on average, from 92 to 114% of the target values. The mean value of the Seronorm 010010 was 13 nmol Hg/L (n = 11; SD, 1) and of the Seronorm 205052 11 nmol Hg/L (n = 5; SD, 1). The recommended value is 15 nmol Hg/L in both batches.

Samples collected from one to three years after amalgam removal.—Hg in plasma and urine was determined in acid-treated samples by the atom fluorescence technique (Corns *et al.*, 1994; Sandborgh-Englund *et al.*, 1998a). The detection limits were 0.1 and 0.5 nmol Hg/L in urine and plasma, respectively. Two commercial reference samples (Seronorm 205052, Nycomed, Oslo, Norway; Lyphochek Quantitative Urine Control-Normal 62031, Bio-Rad Laboratories, Anaheim, CA, USA) were used to check the accuracy. By this method, the Seronorm gave 10

nmol/L (n = 21; SD, 1) and the Lyphochek 59 nmol Hg/L (n = 15; SD, 4). The recommended values are 15 and 60 (range, 50 to 70) nmol Hg/L. To secure the concordance of urine analysis at these low Hg levels, we included in the runs stored samples that had already been analyzed with the atom absorption method. A systematic lower result (20%) was detected, and subsequently, the urine results of the follow-up samples were corrected in accordance with this finding.

Statistical and kinetic analyses

For each subject, the Hg levels in plasma, blood, and urine prior to amalgam removal were calculated as the means of the pre-removal samples. Associations between variables were evaluated by correlation analysis and multiple regression. Confidence intervals for medians were calculated, based on the binomial distribution with the probability 1/2 (Gardner and Altman, 1989). The half-lives of Hg in plasma and urine, after subtraction of the last individually recorded Hg level, were calculated by nonlinear least-squares regression, with weighted least-squares ($1/y_{hat}$). A mono- or bi-exponential model was applied. [The equations used were: $C = A * \exp(-\alpha t) + B * \exp(-\beta t)$, bi-exponential model; and $C = A * \exp(-\alpha t)$, mono-exponential model. α denotes the slope of the first, rapid decline, and β the slope of the second, slow decline.] ANOVA, repeated-measures design, and contrast analysis were used to evaluate the changes of Hg concentrations in blood and plasma in conjunction with the amalgam removal. A p value less than or equal to 0.05 denoted statistical significance.

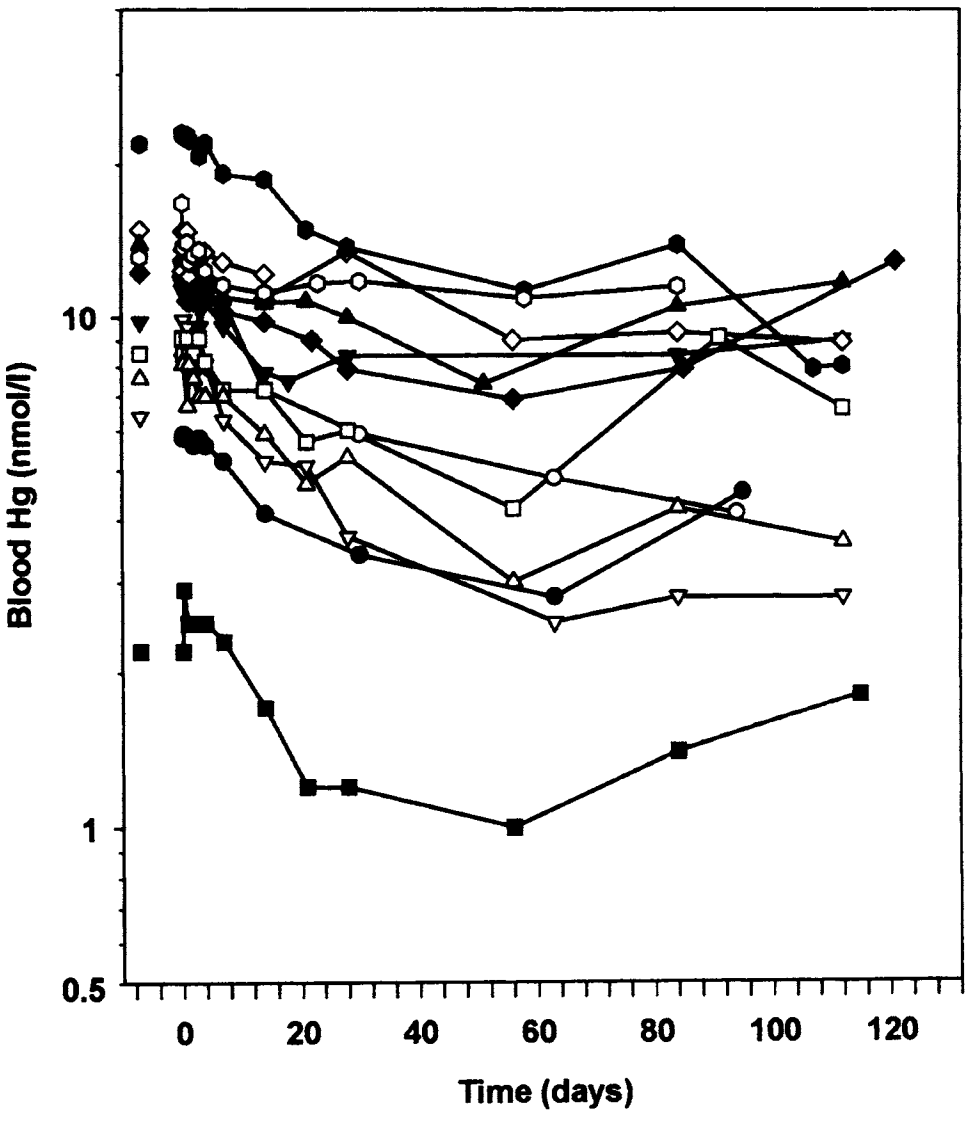

Figure 1. Hg concentration in blood after amalgam removal (Day 0) in 12 subjects. The pre-removal blood Hg concentration is the mean of four samples collected before removal.

Results

Before amalgam removal, the concentrations of Hg in blood and plasma were 12.3 (94% confidence interval, 7.6 to 14) and 3.9 (96% c.i., 2.6 to 7.4) nmol/L, respectively, and the urinary Hg excretion rate was 9.5 nmol/24 hrs (96% c.i., 4.4 to 17) (Figs. 1-3, Tables 2 and 3).

The pre-removal Hg in plasma was significantly correlated to the number of amalgam-filled tooth surfaces (n =10, r = 0.71, p < 0.05). Two subjects who were intense gum-chewers (#101 and 110; Table 2), which is known to cause an increase in Hg levels (Sällsten *et al.*, 1996), were excluded from this correlation analysis. The pre-removal values of Hg in blood, plasma, and urine exhibited a significant interplay (blood *vs.* plasma, $n = 11$, $r = 0.74$, $p < 0.01$; blood *vs.* urine, $n = 11$, $r = 0.81$, $p < 0.01$; and plasma *vs.* urine, $n = 12$; $r = 0.89$; $p < 0.001$).

Removal of the amalgam fillings resulted in a transient increase of Hg in both blood and plasma. Peak levels were recorded between 3 and 48 hrs after the removal, and appeared earlier in blood than in plasma (median, 7 and 24 hrs, respectively). The median increase was 0.7 nmol/L (94% c.i., 0.5 to 2.3) in blood and 1.3 (96% c.i., 0.8 to 2.2) in plasma (Figs. 1 and 2, Table 2). The plasma Hg concentrations obtained within 24 hrs after amalgam removal were

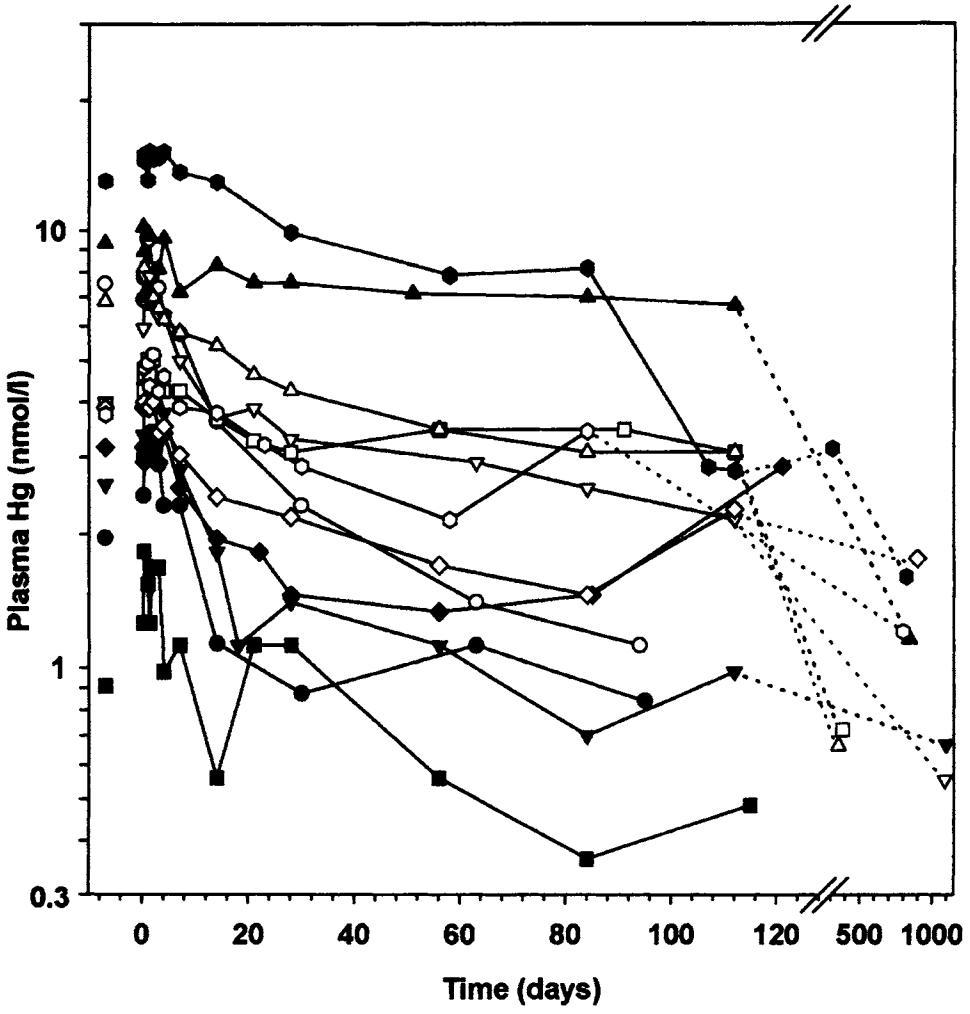

Figure 2. Hg concentration in plasma after amalgam removal (Day 0) in 12 subjects. The pre-removal plasma Hg concentration is the mean of four samples collected before removal. In eight subjects, the plasma Hg concentrations were tracked for up to three years.

significantly higher than those in the pre-removal samples ($F = 11.9$; $p < 0.01$, ANOVA, contrast analysis).

After the peak values, the plasma concentrations declined exponentially. At day 60, the median Hg decrease in plasma was 1.7 nmol/L (96% c.i., 0.8 to 3.4), a 42% reduction (27 to 57%) of the pre-removal level (Table 2). In nine of the subjects, the plasma Hg levels continued to decline or were stable, but in three subjects, an increase was seen between Days 60 and 115 (Fig. 2). The long-term results (from eight subjects) show, on the whole, a continuing decline in plasma Hg. In this subgroup, the median plasma Hg decline at Day 60 was 1.9 nmol/L (93% c.i., 1.1 to 3.4), and up to three years after amalgam removal, the median decline was 3.3 (2.1 to 8.2). The Hg half-lives in plasma in the subgroup were calculated by means of a bi-exponential model, which gave a better fit than a mono-exponential one. The t' of the α-phase ranged from 0.9 to 16 days (median, 4), and of the β-phase from 21 to 121 days (median, 88). Subject #108 was omitted due to poor fit. The coefficients of determination (r^2) ranged from 0.89 to 0.99, indicating an acceptable fit.

In blood, the Hg concentration generally showed the same pattern as in plasma. At day 60, the median Hg decrease was 4.5 nmol/L (94% c.i., 2.3 to 6.6). However, after Day 60, the blood Hg fluctuated considerably in seven subjects (Fig. 1).

There was no apparent transient increase in urinary Hg excretion after the amalgam removal. On Day 60, the median decrease was 4.0 nmol/24 hrs (96% c.i., 2.3 to 9.3), and on Day 85, the corresponding figure was 4.9 (2.2 to 8.9) (Fig. 3, Table 3). In the subgroup of eight subjects followed up to three years, the median decrease in urinary Hg excretion was 5.2 nmol/24 hrs (93% c.i., 2.1 to 8.9) on Day 85, and 9.3 (3.2 to 14) on the last sampling occasion. The half-life of urinary Hg was calculated by means of a mono-exponential model. In one subject (#105), the curve fit was not satisfactory. The median half-life of Hg in urine in the remaining seven subjects of the subgroup was 46 days (range, 35 to 67) (Table 3).

The decrease in urinary Hg excretion 60, 85, and 115 days, and one to three years after amalgam removal was strongly correlated ($r > 0.8$; $p < 0.05$) to the contemporary decrease of plasma Hg concentrations (Fig. 4). Moreover, in eight of the subjects, there were significant correlations ($r > 0.66$; $p < 0.05$) between all contemporary plasma Hg and urinary Hg data (not shown).

There were no significant associations between the transient increase of Hg concentration in blood and plasma and the number of amalgam-filled surfaces, or the duration of the dental procedure. The subsequent declines in blood and plasma Hg concentrations and urinary Hg excretion rate were not significantly associated either with the number of amalgam-filled surfaces or the duration of the dental procedure: Multiple-regression analysis of the decrease in plasma Hg on Day 60 (with age, body weight, number of amalgam surfaces, removal time, pre-removal plasma Hg, and plasma Hg increase as independent variables) revealed a significant effect of age and pre-removal plasma Hg (regression coefficients -0.12 and 0.41, respectively; $p < 0.001$). Multiple-regression analysis of the decrease in urinary Hg excretion on day 60 showed a significant effect of pre-removal urinary Hg excretion only (coefficient 0.41; $p < 0.001$).

Discussion

The present results clearly verify that amalgam fillings are a major source of Hg in biological fluids. Amalgam removal resulted in a minor, transient increase of Hg concentration in blood and plasma, indicating that the removal procedure produced only a relatively limited additional Hg uptake. The lack of correlation between the increase in plasma Hg and the removed number of amalgam surfaces or the duration of the dental procedure may depend on several factors, *i.e.*, the removal procedure was not standardized, the disintegrated amount of amalgam may not be related to the number of surfaces, the subjects had different breathing and swallowing patterns, and the sampling times might not have coincided with the plasma Hg peaks.

The subjects entered the study with a steady-state Hg concentration in plasma, which declined considerably after amalgam removal. There was a large inter-individual variation in the pre-removal levels. The lack of associations between the decreases in blood, plasma, and urinary Hg, on the one hand, and the number of amalgam fillings on the other may be explained by a large inter-individual variation in the impact of amalgam fillings on the pre-removal levels. If the two heavy-gum-chewers were excluded from the multiple-regression analysis, the lack of association remained, in spite of the correlation between pre-removal levels and number of amalgam surfaces. However, an association would probably be demonstrable in a larger study group.

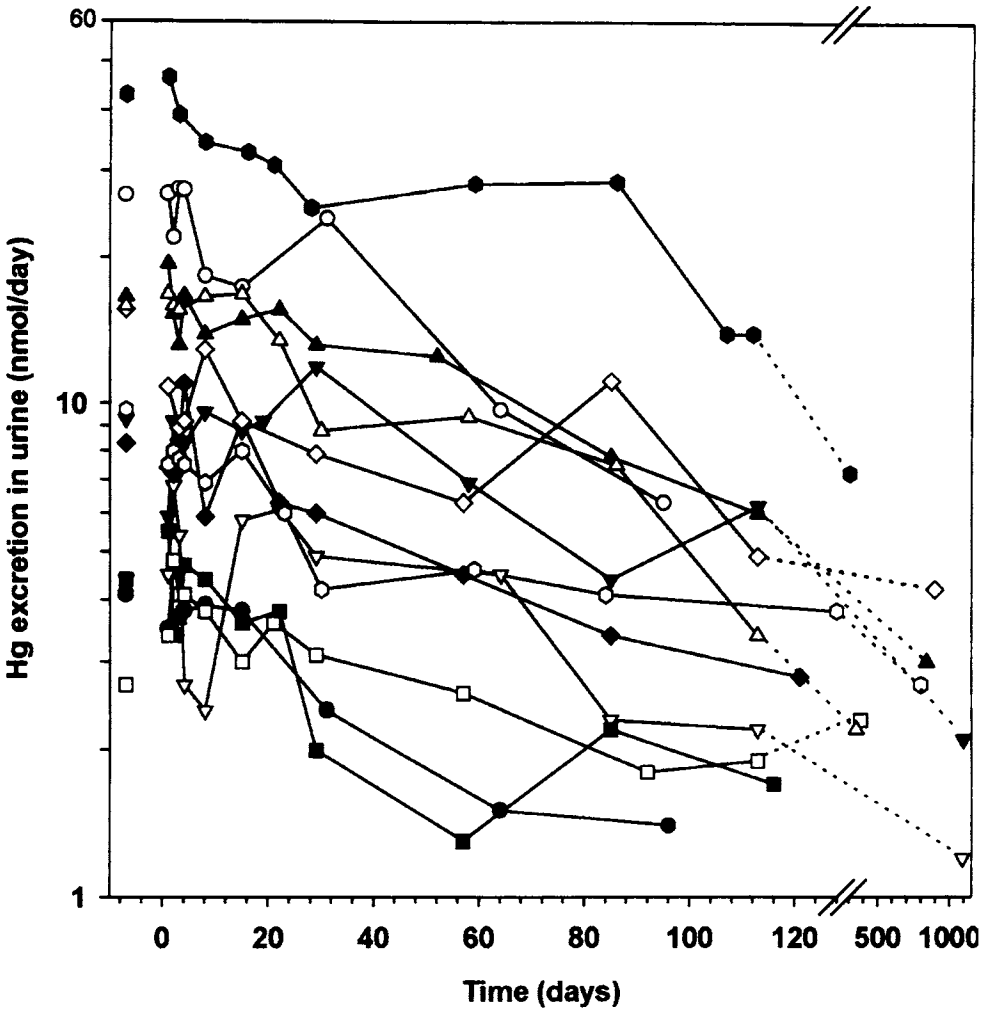

Figure 3. Hg excretion rate in urine after amalgam removal (Day 0) in 12 subjects. The pre-removal urinary Hg excretion rate is the mean of two 24-hour samples. In eight subjects, urinary Hg excretion rates were tracked for up to three years.

Only a few studies on the effect of amalgam removal on blood and plasma Hg concentrations have been published. Molin *et al.* (1990) collected data on Hg in body fluids for one year after amalgam removal in 10 subjects. The mean increase in plasma Hg concentration after amalgam removal was considerably greater, and the mean decline one and two months later was less than in the present study. The participants had more amalgam-filled tooth surfaces, which might partly explain the diversity. The data in the study by Molin *et al.* (1990) are presented on a group basis, and no kinetic analysis was performed. However, evaluation of their group mean data according to a bi-exponential model, after subtraction of the last mean plasma Hg as a baseline (1 yr after amalgam removal), results in a t' estimate of the β-phase of 75 days, which is in good agreement with our median of 88 days. A long half-life of Hg in plasma is in accordance with our observation that plasma Hg continued to decrease after Day 115, and up to three years after amalgam removal, approached the Hg levels found in subjects with no history of amalgam fillings (Sandborgh-Englund *et al.*, 1998b). Snapp *et al.* (1989) reported a median t' of the β-phase of 30 days in blood from eight subjects after amalgam removal. The baseline was assumed to be zero in all cases but one, leading to an overestimation of the half-life. The analysis was performed by curve-stripping, and the conclusions are not supported by the individual plots in all cases.

The kinetics of Hg in blood and plasma in humans has been studied after experimental and occupational exposure. In a study on five healthy volunteers, a radioactive tracer dose of Hg vapor was inhaled, and blood samples were collected for seven days (Cherian *et al.*, 1978). The half-life in plasma and erythrocytes was estimated to be 3.5 and 3.1

Table 2. Plasma Hg (nmol/L) before and after amalgam removal, and estimates of plasma Hg t´ after amalgam removal

	Plasma Hg		ΔPlasma Hg			Half-lives of Hg in Plasma[d]		
Subject	Pre-removal[a]	Increase[b]	Day 60	1 year	2-3 yrs	α	β	r^2
100	1.9	1.2	0.8					
101	7.4	2.0	6.0					
102	2.6	2.5	1.5		1.9[e]	3.6	66	0.99
103	4.0	4.2	1.1		3.5[e]	3.1	104	0.99
104	0.9	0.9	0.4					
105	4.0	1.1	0.5	3.2		0.9	35	0.89
106	3.2	0.2	1.8					
107	3.9	0.1	2.2		2.1[f]	2.4	21	0.96
108	9.3	0.8	2.2		8.2[f]			
109	6.9	1.3	3.4	6.2		7.6	88	0.99
110	13.0	2.2	5.2	9.9	11.4[f]	11	121	0.97
111	3.7	1.4	1.6		2.5[f]	16	90	0.93
Median	3.9	1.3	1.7	6.2	3.0	4	88	
Range	0.9–13	0.1–4.2	0.4–6.0	3.2–9.9	1.9–11.4	0.9–16	21–121	

[a] Mean of four blood collections before amalgam removal.
[b] Maximum increase in plasma Hg 3 to 48 hrs after amalgam removal.
[c] ΔPlasma Hg: Pre-removal plasma Hg subtracted from plasma Hg 60 days, 1, 2, or 3 yrs after amalgam removal.
[d] Half-lives in days. Derived from a two-compartment model: $C = A^*\exp(-\alpha t) + B^*\exp(-\beta t)$; t´ of the α-phase = $\ln 2/\alpha$; t´ of the β-phase = $\ln 2/\beta$. r^2 = proportion of variance explained by the model.
[e] Three years after amalgam removal.
[f] From 2.2 to 2.5 yrs after amalgam removal.

days, respectively; the decay of the isotope prevented further studies on long-term half-lives. Barregård *et al.* (1992) studied the kinetics of Hg in plasma and blood in nine men during 4 to 6 mos after short-term occupational exposure. The last levels measured were used as individual baselines. They found a bi-phasic elimination pattern, and the median t´ of the β-phase was calculated at 15 and 14 days in blood and plasma, respectively. Sällsten *et al.* (1993) studied occupationally exposed subjects during their vacation period, from 17 to 26 days. Due to the short exposure-free period, it was not possible for individual baselines to be established. Instead, median values from an unexposed re-ference group were used. The calculated t´ of the β-phase was 45 days in blood and 36 days in plasma, based on a bi-exponential model with common half-lives, *i.e.*, evaluated on a group basis.

In short, the kinetics of Hg in the human body is not unambiguously clarified. A dose-dependent elimination pattern cannot be excluded. Especially at low-level Hg exposure, it needs to be pointed out that the effect of the baseline estimate on the curve-fitting results is considerable. The influence of MeHg kinetics cannot be ruled out, even when Hg in plasma is studied (see below).

Our study displayed a considerable inter-individual variation in the time of the peak in plasma Hg after amalgam removal. This may be explained by differences in the impact of different forms of Hg (vapor/particulate amalgam) and different routes of absorption (lungs/gastrointestinal tract). Thus, the removal of the amalgam fillings resulted in a substantial increase of Hg excretion in feces 2 days later, the median concentration increasing from 2.7 to 280 µmol Hg/kg dry weight (Björkman *et al.*, 1997). Hence, even a fractionally limited uptake of Hg from the gastrointestinal tract might contribute substantially to the increase in Hg levels in blood and plasma. This factor may contribute to the time lag between exposure and the peak Hg concentrations in plasma.

The decline in Hg concentrations in whole blood, observed in our study, may not be a result of only the removal of amalgam fillings, since decreasing MeHg due to the restrictions in fish consumption (from 30 days before until 60 days after amalgam removal) may have interfered. The subjects did, in normal circumstances, have a moderate intake of fish. Two subjects rarely had any fish, seven had < 2 fish meals/week and three had two or three fish meals/week. Data on normal Hg concentrations in body fluids are sparse, but compared with the tentative reference values reported by Brune *et al.* (1991), the levels of Hg in blood were low even before amalgam removal. The influence of dietary habits (*e.g.*, fish intake) on blood Hg, however, is indicated by fluctuations in blood Hg after Day 60, when the restrictions on fish consumption were lifted.

MeHg in blood is distributed mainly to the red blood cells (WHO, 1990), whereas inorganic Hg is more evenly distributed between red blood cells and plasma (WHO, 1991). The half-life of MeHg has been reported to be between 44 and 79 days (Åberg *et al.*, 1969; Miettinen *et al.*, 1971; Smith and Farris, 1996), and the major fraction is excreted *via* feces (Berlin, 1986). Part of the MeHg is metabolized to inorganic Hg (WHO, 1976). Interpretation of the data reported by Kershaw *et al.* (1980) shows that a rough estimate of the MeHg content in blood can be achieved by subtraction of

Table 3. Urinary Hg excretion rate (nmol/day) before and after amalgam removal, and estimates of urinary Hg t´

	Hg Excretion	ΔHg Excretion[b]		Last Measurement		Half-life of Hg in Urine[d]	
Subject	Pre-removal[a]	Day 60	Day 85	Excretion Rate	Time after Removal[c]	t´	r^2
100	4.1	2.6	2.7	1.4	0.3		
101	27	17	21	6.3	0.3		
102	9.3	2.3	4.8	2.1	3.0	54	0.75
103	4.4	-0.1	2.1	1.2	3.0	42	0.87
104	4.4	3.1	2.2	1.7	0.3		
105	2.7	0.1	0.8	2.3	1.0		
106	8.3	3.9	5.0	2.8	0.3		
107	16	9.3	4.4	4.2	2.5	35	0.69
108	17	4.2	8.9	3.0	2.3	62	0.81
109	16	6.5	8.4	2.2	1.0	46	0.89
110	43	15	14	7.2	0.8	67	0.80
111	9.7	5.2	5.6	2.7	2.2	44	0.80
Median	9.5	4.0	4.9	2.5		46	
Range	2.7–43	-0.1–17	0.8–21	1.2–7.2		35–67	

[a] Mean of two 24-hour samples before amalgam removal.
[b] ΔHg excretion: Pre-removal Hg excretion subtracted from Hg excretion on days 60 and 85, respectively.
[c] Time in years.
[d] Half-life in days. Derived from a one-compartment model: $C = A^*\exp(-\alpha t)$; $t' = \ln 2/\alpha$. r^2 = proportion of variance explained by the model. Note: In subjects 102 and 103, the half-lives are calculated after the peak in Hg excretion (Days 30 and 22, respectively).

the total Hg in plasma from the total Hg in blood. By this means, the MeHg content in blood (before amalgam removal) constitutes a median of 56% (range, 9 to 74%)—the highest levels (> 70%) were found in subjects #102, 106, and 107, and the lowest (< 10%) in subject #109. As a result of the impact from MeHg on blood Hg concentrations, we decided not to use whole blood data for a kinetic analysis.

Hg in plasma has been shown not to be influenced by fish consumption on a group basis (Åkesson *et al.*, 1991). However, it cannot be ruled out that some of the fluctuations in plasma Hg observed after the fish diet restrictions were lifted, *i.e.*, at day 60, may be due to MeHg.

The mechanism of renal Hg excretion is not fully clarified (Zalups and Lash, 1994). It is not understood to what extent the urinary Hg excretion reflects the clearance of Hg from plasma and from the kidney tissue, respectively. Tubular excretion is the major pathway of renal excretion (Berlin, 1986). An equilibrium is presumable between plasma-kidney-urine compartments, supported by the strong associations between Hg in plasma and urine (Fig. 4). The interpretation of urine data is hampered by a substantial between-day variability, and a diurnal variability has been reported (Vokac *et al.*, 1980). In the present study, the mean of the individual between-day variations in urinary flow (CV) was 26% (range, 18 to 42%). The between-day variation in creatinine excretion (CV) was 12% (range, 7 to 22%), which is within the expected range (Cramér *et al.*, 1967) and indicates a very high degree of cooperation by the subjects in the collection of urine.

There are several studies on urinary Hg excretion patterns, both after occupational exposure (Piotrowski *et al.*, 1975; Barregård *et al.*, 1992; Ellingsen *et al.*, 1993; Sällsten *et al.*, 1994), and after removal of amalgam fillings (Molin *et al.*, 1990a; Begerow *et al.*, 1994). The reported urinary Hg half-life ranged from 40 (Barregård *et al.*, 1992) to 95 days (Begerow *et al.*, 1994). The levels of Hg exposure and the methods for data handling and calculation differ among the studies. In most studies of occupationally exposed subjects, a baseline level was subtracted prior to the curve fit and half-life estimate, which seems appropriate due to the fact that the subjects presumably had a background exposure from their amalgam fillings. However, even in subjects without any history of amalgam fillings, Hg is detectable in the urine; observations from our laboratory have verified a daily excretion of 0.2 to 3 nmol (Sandborgh-Englund *et al.*, 1998b). This may originate from other sources if inorganic Hg exposure, as well as MeHg, metabolized to inorganic Hg (WHO, 1990; Smith and Farris, 1996). The fact that Begerow *et al.* (1994) did not subtract individual baselines may explain their high value of 95 days. A urinary half-life of 58 days is calculated from the group mean values on Hg excretion, after subtraction of the last-measured Hg excretion (Molin *et al.*, 1990), one year after amalgam removal. This agrees fairly well with the median of 46 days found in the present study.

While an increase in urinary Hg excretion after amalgam removal has been reported (Molin *et al.*, 1990; Begerow *et al.*, 1994), no such increase was observable in the present study. It is probable that the additional exposure by amalgam removal, found in the present study, was too limited, and that a possible increase was overshadowed by the normal variations and imprecision in determinations at these low levels. In two subjects, however, the Hg excretion pattern was peak-shaped (#102 and 103). Notably, these subjects also exhibited the greatest increase in plasma Hg, suppor-

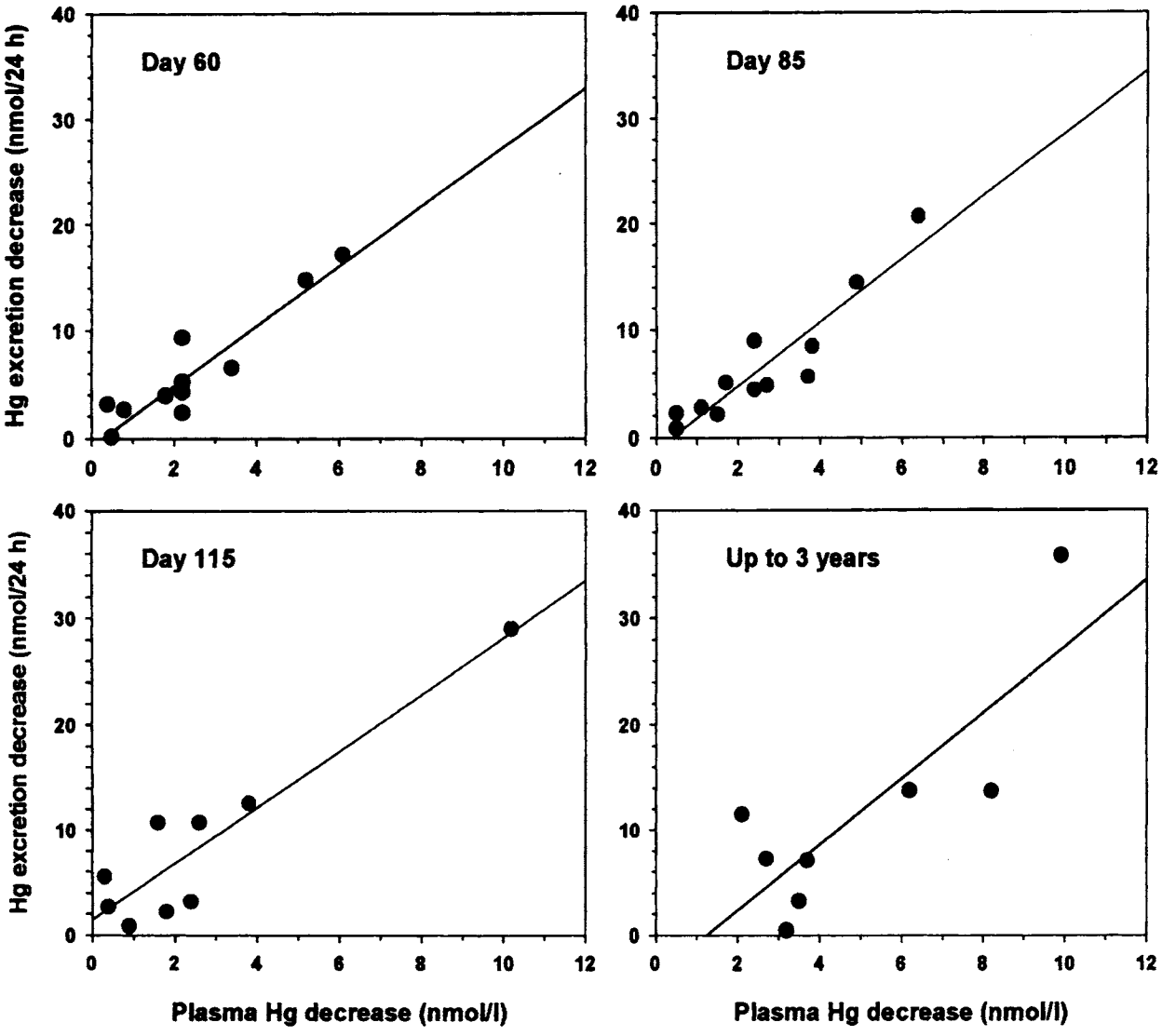

Figure 4. The associations between the decline in urinary Hg excretion and decline in plasma Hg. The pre-removal Hg levels are subtracted from the Hg levels at Days 60, 85, and 115 and one to three years after amalgam removal. The correlations are: (Day 60) $n = 12$, $r = 0.92$, $p < 0.001$; (Day 85) $n = 12$, $r = 0.93$, $p < 0.001$; (Day 115) $n = 9$, $r = 0.93$, $p < 0.001$; and (after 1–3 yrs) $n = 8$, $r = 0.82$, $p < 0.05$.

ting the supposition that the peak in urinary Hg excretion was caused by the Hg dose from amalgam removal.

The change in urinary Hg excretion can be used as a reflection of the previous daily uptake from amalgam fillings. The ΔHg excretion was (median) 9.3 nmol/24 hrs (range, 0.4 to 36; equal to 1.9 and 0.1 to 7.1 μg/24 hrs) up to 3 yrs after removal. This is in the lower region of the estimates reported earlier (WHO, 1991; US Public Health Service, 1993). However, fecal excretion is also an important route of systemic excretion, and thus the present estimate of the daily absorbed Hg dose from dental amalgam is too low. The fraction of fecal Hg excretion is poorly documented, but is indicated to be about 1/3 (deduced from Björkman *et al.*, 1997).

In the present study, two different methods of Hg analysis were used. The agreement between the CVAAS and CVAFS methods is considered to be high, as judged from analysis of the Seronorm samples. The more recent method resulted in a systematically 20% lower recovery of Hg in urine, as indicated from the re-analysis of stored samples, which might be explained by the differences in sample treatment prior to analysis.

In conclusion, our results show that the removal of amalgam fillings results in a minor increase of Hg in plasma, followed by a considerable decline in all media. The Hg levels in plasma and urine slowly approached the levels found in subjects without any history of amalgam fillings.

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