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MERCURY METABOLISM AND SELENIUM PHYSIOLOGY STUDIES

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Project Description

This work examines the influence of dietary mercury on selenium physiology and the influence of dietary selenium on mercury metabolism.¹ Studies have been performed in rats to compare the effects of dietary exposure to a range of molar ratios of mercury and selenium. The effects of mercury and selenium at these varied molar ratios were followed in rats fed selenium-deficient, selenium-adequate, or selenium-rich diets, and the signs and symptoms of mercury toxicity were compared to the molar ratios of mercury and selenium occurring in vulnerable tissues of the nervous system. Selenium's effects on mercury distribution in these tissues was compared to its influence on mercury concentrations in hair and blood since these materials are used as indicators of exposure.

These studies are augmented by our collaboration with the Seychelles Child Development Study which is examining the relationship between methylmercury exposure from maternal fish consumption and potential development of neurodevelopmental toxicity in children. Whole blood samples collected from Seychellois mother and child pairs at delivery were analyzed for their selenium contents and will be evaluated in relation to mercury contents, maternal fish consumption, and the children's development.

Uniting the results of these studies, preliminary development of a computational model of mercury and selenium distribution into tissues has been initiated in this study. The model incorporates knowledge of normal tissue selenium distributions and measured concentrations of mercury and selenium present in tissues of animals exposed to known amounts of these elements. This tool is being applied in a basic examination of the time and concentration-dependent accumulation of methylmercury and its potential influence on selenium bioavailability. Comparisons and evaluations through use of the computational model of mercury-selenium interactions examines their mass action effects at the molecular level and observes the effects of mercury-dependent disruptions of selenium availability at the organism level.

¹ Since selenium and mercury both occur in a multitude of molecular forms that continually and, in some cases, repeatedly interconvert, it is inappropriate and inaccurate to attempt to use terms other than "mercury" and "selenium" to designate their occurrence. When specific molecular forms are discussed, they will be named, otherwise it should be understood that the comprehensive presence of these numerous chemical forms are all-inclusively designated by the use of the name of the element.

This study is also examining trace elements in human hearts removed during transplantation procedures performed on patients with dilated cardiomyopathy (DCM). An earlier study indicated many times more mercury and antimony were present in myocardial tissue from these patients than is present in tissues from control subjects. While the earlier study used needle biopsy samples of heart tissue, this study will be able to collect substantially larger samples from explanted hearts. This will minimize analytical defects such as contamination and signal artifacts that might have affected previous studies.

Goal

These studies are intended to improve understanding of the biochemical basis of the influence of selenium on methylmercury toxicity and the effects of mercury toxicity on selenium physiology. The goal of the invitro study was to evaluate binding behavior between mercury and selenium. The goals of the in vivo study were to examine the influence of dietary methylmercury intake on selenium distribution in hair, blood, and brain and explore the effects of dietary selenium intake on mercury distribution and the relationship between their molar ratios and development of signs of mercury toxicity. The goal of the population study of blood selenium concentrations in Seychellois mothers and children was to accurately evaluate the effects of fish consumption on selenium status in relation to their mercury exposure. The goal of the pathological study of mercury, antimony, and selenium concentrations in diseased hearts will be to quantitatively assess mercury, antimony, and selenium in tissues collected from control and DCM patients.

Rationale

Although the physiological consequences and manifestations of methylmercury toxicity have been described (1–5), the direct molecular mechanisms responsible for the various physiological perturbations reported have not been well defined. However, it has been recognized for almost 30 years that physiological consequences of MeHg exposure may relate to impairments of selenoenzyme synthesis or activities (6). The mechanism of selenium-dependent protection against methylmercury toxicity and the mechanism of mercury toxicity itself converge in this hypothesis (Figure 1).

Evidence in support of this hypothesis is growing. For instance, although lipid peroxidation has long been noted to accompany Hg toxicity, it has only recently been recognized that Hg does not promote the direct nonenzymatic lipid peroxidation as copper and iron do (7). Instead, inorganic mercury (Hg^{2+}), but not methylmercury, inhibit selenium-dependent glutathione peroxidase activity, particularly when selenium availability is low. Since intracellular methylmercury is gradually demethylated to release Hg^{2+} that may subsequently sequester selenium, this mechanism has the additional virtue of potentially explaining the prolonged latency period that accompanies exposure to toxic amounts of methylmercury (8). If the postulated mechanism is correct, it would also explain why methylmercury exposure severely compromised selenoenzyme activities in tissues of neonatal experimental animals whose dams were fed low dietary Se, but not in tissues of neonates whose dams were fed selenium-enriched diets (9, 10).

It may be that sufficient selenium must be available to offset the quantity lost to mercury-binding and support synthesis of selenoenzymes in order to prevent onset of physiological consequences of methylmercury exposure. Since selenoenzymes are present in all cells of all animals, it is possible that the reason selenium-dependent protection against mercury toxicity has been observed in all species studied is due to the apparent necessity of sustaining selenoenzyme synthesis in brain and neuroendocrine tissues (11–15). The known functions of these enzymes include free-radical detoxification and thyroid hormone

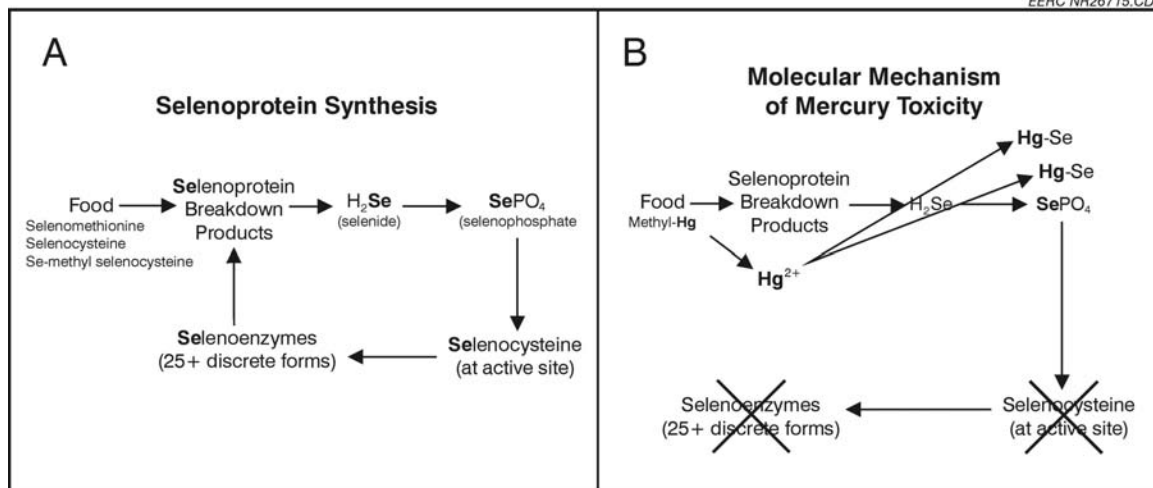


Figure 1. Normal selenoenzyme synthesis (Panel A) and hypothesized mechanism of mercury toxicity (Panel B).

metabolic regulation among a number of other significant activities, but the functions of many of these enzymes remain uncharacterized (16). Loss of selenoenzymes and their functions as a result of mercury-dependent sequestration of selenium could explain many of the pathological effects of mercury toxicity. These include peroxidative damage, altered glutathione metabolism, and disruptions in signal transduction. Other currently unrecognized selenium-dependent functions may be responsible for other aspects of the pathological effects of methylmercury toxicity. Impaired selenoenzyme activities have been found to accompany mercury toxicity (6, 10, 17–19). Loss of selenoenzyme activities is certainly a contributing cause of the pathologic consequences associated with mercury toxicity and may be the primary molecular mechanism of mercury toxicity (10, 20).

How mercury and other heavy metals occur in biological tissues is of significant interest for understanding the toxicological behavior of the heavy metal and the ramifications of environmental exposures to such metals. Even though such elements may only be present in the body at low levels, they are often significantly concentrated in certain organs. This is especially true for marine mammals and birds, whose diets may consist of fish contaminated with such heavy metals. Liver concentrations of Hg in excess of 2000 ppm have been reported for northern fur seals (21). Mercury accumulates in the liver over the lifespan of the animal. However, despite such high accumulations of heavy metals in key organs, the animal often shows no adverse signs of heavy metal poisoning, and it has been suggested that the mercury accumulates in the liver in a minimally hazardous inorganic form rather than as a more toxic organomercury species. It had been noted that Se concentrations were often high in the same tissues in which the Hg content was also high, leading to the suggestion that formation of HgSe may be responsible for the lack of toxicity. Recent work using x-ray absorption fine structure (XAFS) spectroscopy by a Japanese group (22) has confirmed that the mercury in liver tissues of the northern fur seal and the black-footed albatross is present as granules of HgSe or a solid solution of HgS and HgSe, Hg(S,Se). The relative inertness, insolubility, and immobility of such compounds in key organs prevent the heavy metal from having a serious toxic effect on the animal despite their likely accumulation over the lifetime of the animal.

In the United States, approximately 43% of all heart transplantations are performed for DCM a progressive pathology that appears to occur secondary to myocarditis (23). A 1999 report on trace elements in tissues obtained from patients with idiopathic dilated cardiomyopathy (IDCM) indicated

>10,000 times more mercury was present in their myocardial tissue than in their skeletal muscle (24). Mean mercury concentrations in endomyocardial tissue was 22,000 times higher in IDCM patients (178.5 mg/g) than it was in control subjects (0.008 mg/g). Antimony concentrations were also found to be remarkably higher in cardiac tissues from these same patients. Of the 30 other elements measured in the test and control groups, no other elements showed evidence of such unusually high trace element accumulation. The accumulations of mercury and antimony showed strong correlations with the severity of the pathological symptoms observed in these patients. However, these extraordinarily high elemental accumulations are difficult to understand since no unusual exposure risk factors for either of these elements were noted among any of these patients.

The present study applies a multiperspective approach to examining the hypothesis that the molecular mechanism of methylmercury toxicity may be the result of mercury-dependent selenium sequestration. Formation of highly insoluble complexes of mercury selenides impairs the bioavailability of selenium, leading to loss of intracellular selenium bioavailability and impaired synthesis of selenium-dependent enzymes. This postulate is linked to the hypothesis that the mechanism of selenium's well recognized protection effects against methylmercury toxicity is the result of supplying sufficient selenium to offset losses to mercury sequestration and support sustained selenoenzyme synthesis. The studies of this project are intended to test these hypotheses, and establish the effects of selenium on hair and blood indices that are used to evaluate mercury exposure.

Approach

Objective 1

Selenium concentrations are often high in the same tissues in which mercury contents are high, leading to the suggestion that formation of relatively inert HgSe is responsible for their coaccumulation. To directly assess HgSe formation in a physiologically appropriate mammalian model of the results of methylmercury and selenium from fish consumption, we examined liver tissues of a beluga whale and a pygmy sperm whale. These samples were collected from stranded whales and provided courtesy of the National Oceanic and Atmospheric Administration. XAFS spectroscopy of the L_{III}-edge of mercury and the K-edge of selenium was used to identify possible Hg and Se species.

Objective 2

Mercury has an extraordinarily high affinity for selenium, thus exposure to mercury can result in sequestration of selenium within cells of vulnerable organs. At high dietary mercury concentrations, formation of these insoluble mercury selenides may result in diversion of selenium from selenoprotein synthesis and diminishment of selenoenzyme activities. Our objective was to investigate the effect of dietary mercury on selenium distributions and selenoenzyme activities in rats fed diets containing varying concentrations of methylmercury and selenium. Based on nutrition studies of selenium, diets that were selenium-deficient (0.1 $\mu\text{mol Se/kg}$; ~ 0.01 ppm Se), selenium-adequate (1.25 $\mu\text{mol Se/kg}$; ~ 0.1 ppm Se) or selenium-rich (25 $\mu\text{mol Se/kg}$; ~ 2 ppm Se) were employed. These were supplemented with either no methylmercury (0 $\mu\text{mol Hg/kg}$; 0 ppm Hg), low methylmercury (2.5 $\mu\text{mol Hg/kg}$; ~ 0.5 ppm Hg), or high methylmercury (75 $\mu\text{mol Hg/kg}$; ~ 15 ppm Hg). To monitor methylmercury toxicity-dependent effects on weight gain and selenium-dependent protective effects against this sign of methylmercury toxicity, rat body weights were measured weekly and plotted relative to their time on the diets. During the final week

of the study, food consumptions of the treatment groups were measured. On Day 63 of the study, rats were anaesthetized, hair samples for Objective 2 were collected, and blood and organ tissues were collected for mercury and selenium analysis.

Objective 3

Conflicting observations and conclusions have arisen from the ongoing studies of mercury-dependent health effects in the Faroe Islands and in the Seychelles Islands. While researchers in the Faroe Islands reported neurological defects in children exposed to low levels of mercury in the womb, the Seychelles study has found no adverse effects from prenatal methylmercury exposure, even at levels of exposure 10–20 times higher than what is common in the United States. In further contrast, maternal fish consumption in the Seychelles correlated with an improved neurodevelopmental outcome in some indices. The discrepancies between the observations and conclusions reported in these studies may be due to dietary differences in the study populations. Selenium's protective effect against mercury toxicity may be one dietary factor but had not been assessed. The objective of this study is to include selenium analysis in the ongoing Seychelles Study. Selenium and mercury analysis are being included as concomitant variables regarding neurodevelopmental assessment end points for the children born to mothers with known exposures to methylmercury from fish. We measured selenium in whole blood samples collected from ~250 maternal–fetal pairs from the current Seychelles study. These values will be assessed in concert with dietary records from the individual subjects and analytical data reflecting blood mercury contents. The blood concentrations of omega-3 fatty acids, mercury, and selenium will be examined in relation to neurodevelopment assessed in the children. The children are currently approaching or recently reached 2 years of age, and their mental development is currently being monitored.

Objective 4

The samples originated from needle biopsies of heart tissue that were collected from the patients, amounting to approximately 1–3 mg of tissue (wet weight). Although this is sufficient tissue mass for quantitative determinations by neutron activation analysis, such small samples are more prone to analytical defects such as contamination and signal artifacts. Larger tissue sample sizes would provide more robust analytical data, but were unavailable in the Italian study. Trace element concentrations in heart ventricular tissues obtained from patients suffering from dilated cardiomyopathy will be compared to similarly collected tissues from patients diagnosed with ischemic cardiomyopathy and a noncardiomyopathy control group.

Progress/Status

The successful identification and quantification of HgSe occurring in whale liver tissues has encouraged us to perform further analysis of predatory whale tissues. Since these are mammalian species that are exposed to methylmercury as a result of fish consumption, they appear to be a highly suitable model for expected biochemical and physiological effects of fish consumption on eventual distribution of mercury and selenium. A resource for obtaining a panel of brain and neuroendocrine tissues has been identified, and these tissue samples are available for elemental analysis and HgSe characterization.

Hair and tissue samples that were collected from the rat study have been analyzed for mercury and selenium, and the molar ratios for mercury and selenium present in the tissues have been compared and plotted. Correlations have been determined between the dietary treatment effects on observed mercury and selenium distributions in the analyzed tissues. The results from this study and follow-up studies whose design was based on results from this work have been presented at several regional, national, and international meetings. Several aspects from this work will be presented at Mercury 2006, the biannual

international conference on this subject. Analysis of selenium and mercury contents of pituitary tissues and selenoenzyme activities of the brain are currently under way and will be evaluated in relation to dietary treatment as well as to hair and blood analytical results. A manuscript describing the results of this study is in preparation for submission for publication.

The maternal and cord blood samples collected from babies born in the Seychelles have been analyzed for selenium and have been entered into the study database at the University of Rochester. These selenium analyses will be used to assess beneficial effects of fish consumption upon neurodevelopmental end points being measured in the growing children. The results from this study have been presented at several regional and national meetings and will be included in the platform presentation to be made at the International Mercury Meeting. A manuscript describing the results of this study is nearing the final stages of preparation for publication.

The measurement of mercury and selenium in heart tissues was delayed because contrary to expectations of even internationally prominent heart researchers, no heart tissue repository of fresh frozen samples appears to exist in the United States or Europe. As a result of this project's urging, a frozen heart tissue collection has been initiated at the Mayo Clinic, and heart tissue samples are now being collected for elemental analysis.

Results

In Vitro Study: Examination of Direct Interactions between Mercury and Selenium

Mercury L_{III} and L_{II} XANES spectra of the whale liver samples were compared to spectra for elemental mercury, forms of mercuric oxide, mercuric nitrate, mercuric sulfide, and mercuric selenide. The signal/noise ratio for the L_{II} edge was found to be somewhat weaker and the resolution of features at the L_{II} -edge is not quite as good as that at the L_{III} -edge. Therefore, XAFS spectra were obtained at the L_{III} -edge of mercury. For the beluga whale liver tissues, in which the mercury content was 150 ppm (lyophilized tissues) and the Hg/Se molar ratio was 0.66, HgSe was clearly present as indicated by both the mercury and the selenium XAFS spectra. For the pygmy sperm whale liver tissues, in which the mercury content is much lower (9 ppm in lyophilized tissues) and the Hg/Se molar ratio was also much lower (0.17), the mercury XAFS spectrum was similar to that of the beluga whale, but the selenium XAFS spectrum was much different, indicating that proportionately less selenium was associated with mercury as HgSe. Qualitatively, the mercury XAFS data indicate that HgSe is the major form of mercury in the beluga whale samples. Similarly, the data for mercury in the pygmy whale, although significantly noisier compared to the corresponding data for the beluga whale because of the lower total mercury content, also indicates that much of the mercury in that whale's liver is present as HgSe.

Based on the chemical analysis of mercury and selenium in the two whale liver samples, the molar ratio of Hg:Se is less than 1.0 for both, implying that there is an excess of selenium over that needed to form HgSe and sufficient to maintain selenium-dependent enzyme activities. Whereas HgSe constitutes about 67% of the total selenium in beluga whale liver, it is only about 17% of the total selenium in pygmy sperm whale liver. Hence, a significant fraction of the selenium is likely to be present in other forms in the case of the pygmy sperm whale. Such differences are confirmed by the selenium XANES data shown in Figure 2.

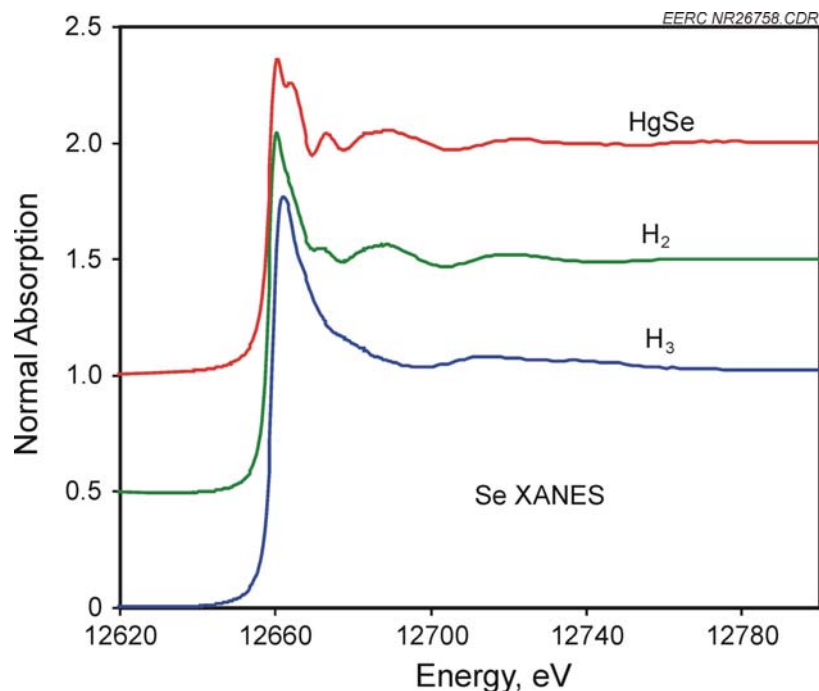


Figure 2. Se K-edge XANES data for HgSe and for beluga (H2) and pygmy sperm whale (H3) liver.

While various prominent features of the selenium XANES spectrum of HgSe are clearly apparent in the spectrum of the beluga whale liver (H2), they are not obvious in the corresponding spectrum of the pygmy sperm whale liver (H3). These differences are consistent with the analytical data for mercury and selenium in the two liver samples.

In Vivo Study: Interactive Effects of Dietary Methylmercury and Selenium in Rats

One of the more prominent and well-known signs of mercury toxicity in experimental animals is depressed growth. Figure 3 depicts the mean μ standard deviations for the weights of rat groups fed diets that varied in mercury and selenium contents at weekly intervals during the course of the 9-week study. The weights of rats fed selenium-deficient, selenium-adequate, or selenium-rich diets are shown for the control groups fed these diets without added methylmercury and treatment groups fed these diets with methylmercury added at 75 $\mu\text{mol MeHg/kg}$ diet.

Rats fed diets with high (75 $\mu\text{mol MeHg/kg}$; 15 ppm) mercury concentrations gained less weight than rats fed diets containing 0 or 2.5 $\mu\text{mol MeHg/kg}$ (0.5 ppm) mercury (Figure 3A). Among rats fed a selenium-adequate diet (1.25 $\mu\text{mol Se/kg}$; 0.1 ppm), growth of the group fed the highest mercury concentration tended to be slightly depressed (Figure 3B), but not nearly so much as was apparent in the selenium-deficient study group. The protective effect of selenium was even more apparent among rats fed selenium-rich diets (Figure 3C) where there was no indication of mercury-dependent growth depression.

The hair samples collected at the end of Week 9 have been analyzed, and the results shown in Figure 4 depict their Hg and Se contents. The results show a positive linear relationship between mercury consumption and hair mercury levels. Hair color and selenium status did not have a major influence on hair mercury deposition in black or white hair from Long Evans rats. Ethnicity and genetic variables

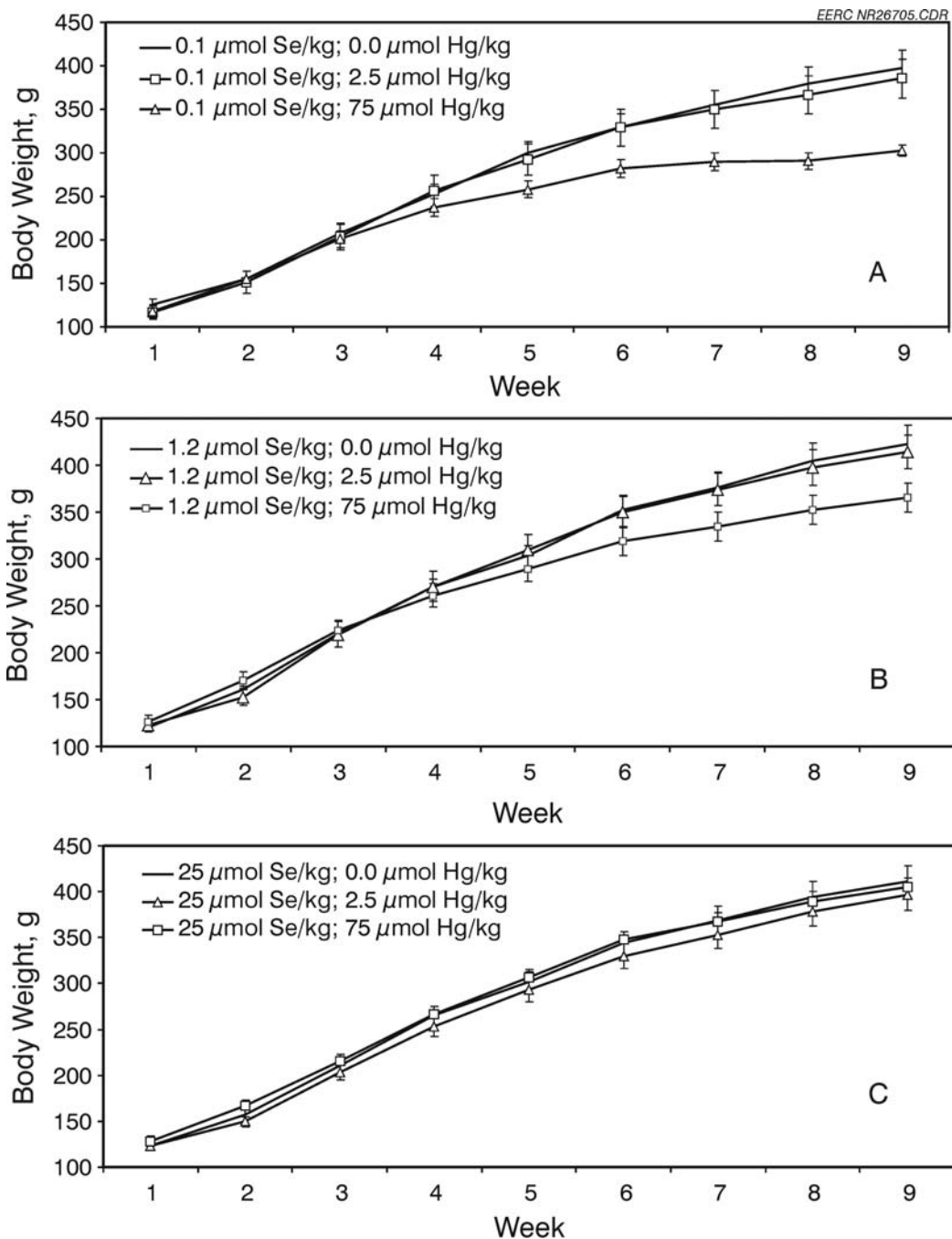


Figure 3. Effects of dietary methylmercury and selenium on weight gain in growing rats.

displayed in human hair composition and texture cannot be adequately studied in animals, but the results of this study suggest that differences reported between hair colors in humans are not likely to be directly related to hair color itself. Controlled human studies need to be performed to verify if compositional differences that accompany distinctions in hair color in humans has an influence on distribution of mercury into hair. However these data provide evidence that hair color is not a contributing factor that needs to be considered in future animal studies.

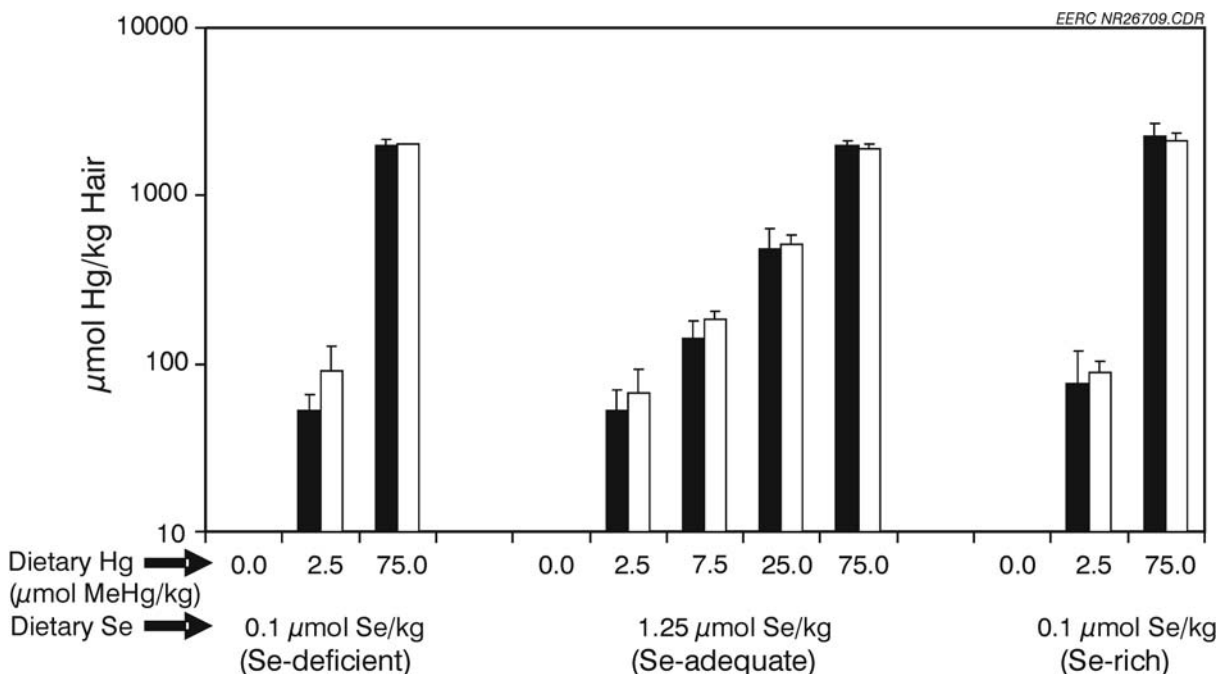


Figure 4. Effects of dietary methylmercury and selenium on Hg in black and white hair.

The blood samples collected at the end of the study have been analyzed for mercury and selenium (see Figure 5A and B). Figure 5A depicts the effects of diet on mercury and selenium concentrations (mean \pm SEM) in blood samples collected from the treatment groups. Normal blood selenium (from rats fed adequate dietary selenium) was $8.6 \pm 0.3 \mu\text{M}$ (mean \pm SEM). Although all rats were selenium adequate at the start of the study, after 9 weeks on the selenium-deficient diet blood Se had diminished $\sim 93\%$ to $0.6 \pm 0.1 \mu\text{M}$, a concentration proportionally similar to the relative Se concentrations in their diets. Blood selenium in rats fed selenium-enriched diets almost doubled, rising to $16.1 \pm 2.0 \mu\text{M}$. However, in this case the twofold increase in blood selenium does not proportionally reflect the 20-fold greater concentration of selenium in the blood. This effect is well recognized and appears to be the result of the tight physiological regulatory processes that regulate selenium. The primary means of eliminating excess selenium is through forming dimethyl- and trimethyl-selenides that exit the body through urinary excretion and exhalation.

Dietary selenium exerted an influence on the distribution of mercury in blood. Compared to blood mercury from selenium-deficient rats, mercury concentrations in rats fed 2.5- $\mu\text{mol MeHg/kg}$ diets increased by $\sim 50\%$ when dietary selenium was adequate or enriched. Among rats fed 75 $\mu\text{mol MeHg/kg}$, blood mercury was $\sim 30\%$ higher in the rats fed adequate selenium than in rats fed deficient selenium and $\sim 40\%$ higher in rats fed selenium-enriched diets.

Meanwhile, consumption of dietary methylmercury at 75 $\mu\text{mol MeHg/kg}$ diminished blood selenium concentrations in selenium-deficient and selenium-adequate rats by 32% and 10%, respectively. Blood selenium in selenium-deficient and selenium-adequate rats was also lower in rats fed 2.5 $\mu\text{mol MeHg/kg}$, although the declines were much less (10% and 5%, respectively). In contrast, selenium concentrations in rats fed selenium-rich diets were increased by $\sim 40\%$ when 2.5 or 75 $\mu\text{mol MeHg/kg}$ was present in the diet, possibly as the result of mercury-selenium complex formation and retention of the insoluble precipitates.

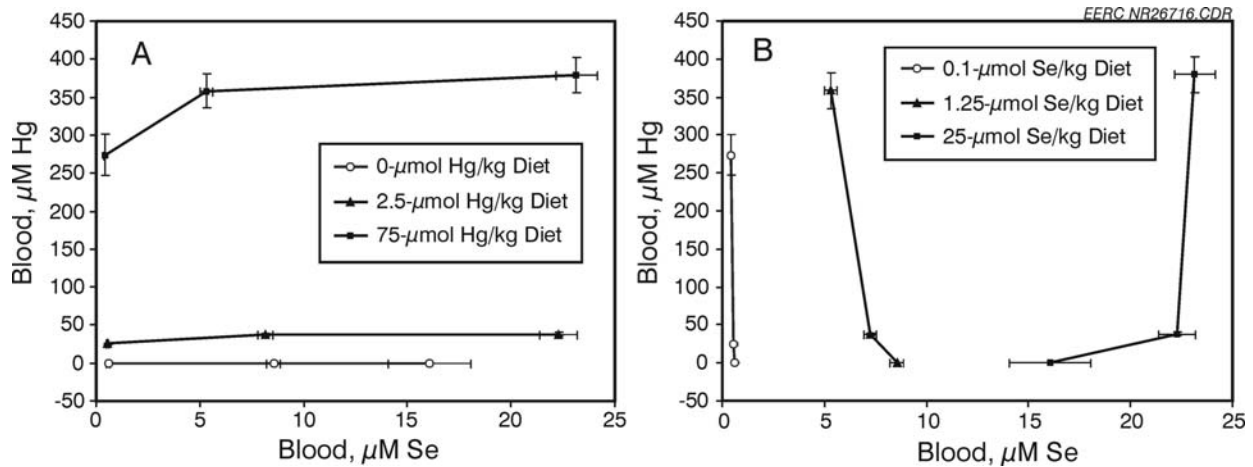


Figure 5A and B. Effects of dietary methylmercury and selenium on blood mercury and selenium contents.

Brains collected from the rats at the end of the study were cut in half between hemispheres, one portion was retained in storage at -80°C for enzyme analysis, and the other half was freeze-dried and the homogenized powder used for elemental analysis. The concentrations of mercury and selenium present in brains from the dietary treatment groups (mean \pm SEM) are depicted in Figure 6A and B. The selenium concentration in brains of rats fed selenium-adequate diets was $10.9 \pm 0.3 \mu\text{mol Se/kg}$. Brain selenium concentrations are known to be remarkably well controlled and, as expected, homeostatic regulation was apparent in this study. After 9 weeks on a selenium-deficient diet, brain selenium concentrations had diminished only 14%, even though the selenium in the blood supplying the brain had diminished 93%. Rats fed selenium-enriched diets containing 20-fold greater selenium displayed a similar but even more tightly controlled homeostatic regulation than was evident in the blood, since brain tissue selenium contents increased by only $\sim 30\%$ relative to rats fed adequate dietary selenium.

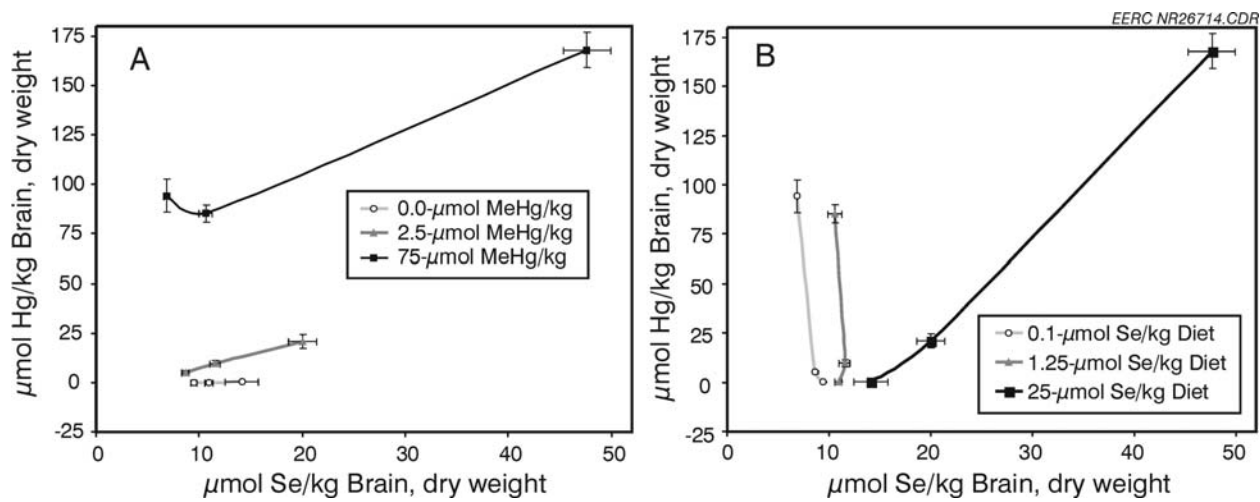


Figure 6A and B. Effects of dietary methylmercury and selenium on brain mercury and selenium contents.

Selenium-deficient rats challenged with dietary mercury were unable to maintain their selenium concentrations. Dietary methylmercury fed at 2.5 $\mu\text{mol MeHg/kg}$ slightly diminished brain selenium contents to 80% of normal. However, brain selenium concentrations in rats fed 75 $\mu\text{mol MeHg/kg}$ dropped to ~60% of normal, about equal to the lowest level of brain selenium ever achieved in studies that had consistently fed selenium-deficient diets to rats for as many as 16 generations. Rats fed selenium-adequate diets were able to maintain their brain selenium concentrations at near-normal levels regardless of the level of dietary methylmercury they were challenged with. However, selenium concentrations in brains of rats fed selenium-enriched diets doubled when they were fed 2.5 $\mu\text{mol MeHg/kg}$ and were about four times greater than normal when they were fed 75- $\mu\text{mol MeHg}$ diets.

The influence of dietary selenium on mercury accumulation in the brain was also dramatic. Mercury contents in brains of rats fed diets containing 2.5 $\mu\text{mol MeHg/g}$ were $5.1 \pm 0.7 \mu\text{mol Hg/kg}$ in rats fed selenium-deficient diets, but almost twice as high when rats were fed selenium-adequate and four times greater when fed selenium-enriched diets. Mercury contents in brains of rats fed diets containing 75 $\mu\text{mol MeHg/g}$ were $94.3 \pm 8.3 \mu\text{mol Hg/kg}$ in rats fed selenium-deficient diets, and were unchanged when rats were fed selenium-adequate diets. However, brain mercury contents almost doubled among rats fed selenium-enriched diets.

Assessing mercury toxicity on the basis of failure to thrive, the selenium-deficient rats fed the high level of dietary mercury were the most affected and the rats fed selenium-enriched diets were the least intoxicated. However, the mercury concentration in the brains of rats fed selenium-deficient diets were only half as great as that of rats fed selenium-enriched diets. Obviously, if mercury toxicity was a simple concentration-dependent process, the results would be the opposite of what was observed.

The “tonic” hypothesis suggests selenium’s protective effect against mercury toxicity is the result of selenium forming insoluble complexes with mercury to prevent it from interacting with or damaging biologically significant molecular species within vulnerable tissues such as the brain. However, since the amount of mercury present in the brains of rats is in greater absolute molar excess in the group fed selenium-enriched diets (~120 μmol more Hg is present than Se) than in the brains of rats fed selenium-deficient diets (~90 μmol more Hg is present than Se), it does not appear that selenium’s protective effect occurs as a result of “tonic” activity.

Examining the molar Hg:Se ratios in the brains of rats provides illuminating insights into the nature of selenium’s protective effect against mercury toxicity. In brains of rats fed selenium-deficient diets with high dietary mercury, the Hg:Se ratio was ~14. In brains of rats fed high dietary mercury along with selenium-adequate or selenium-enriched diets, the brain Hg:Se ratios were ~8 and 4, respectively. Since none of these ratios indicate sufficient selenium was available to sequester more than a fraction of the mercury present, the mechanism responsible does not involve selenium-dependent sequestration of mercury. However, based on the great increases in both mercury and selenium concentrations in the brains of rats fed diets containing high concentrations of mercury and selenium, mercury selenides appear likely to be forming.

The high molar ratio of Hg:Se in selenium-deficient rats associated with development of signs of mercury toxicity may be the combined result of the profound deficiency of selenium in their brains. Not only is the total selenium reduced to only 60% of normal, a significant fraction of what little selenium is present is undoubtedly bound to mercury. Analysis of selenium-dependent enzyme activities will be performed on the brain samples from this study, but support of selenium-dependent enzymes appears likely to be severely compromised under these conditions. In selenium-deficient rats that were not challenged with dietary methylmercury, the body was able to mobilize and deliver sufficient selenium from body stores to maintain a more normal selenium concentration. However, challenges with dietary

methylmercury appears to impair selenium mobilization from accessible pools in the body and or transport and delivery of selenium to the brain.

Meanwhile, in rats provided selenium-enriched diets, the increased accumulation of mercury in the brain was answered by increased selenium accumulation. Formation of mercury selenides may explain why both mercury and selenium accumulation increased dramatically in brains of methylmercury-exposed rats that were fed selenium-enriched diets. Since these rats did not show signs of mercury toxicity, sufficient amounts of selenium from their daily dietary intakes may be maintaining selenium-dependent enzyme activities in their brain tissues.

Population Study: Selenium in Blood Samples from Seychellois Mother–Child Pairs

As an indication of nutritional selenium status, whole blood is superior to plasma or serum. Plasma and serum selenium concentrations are approximately equivalent to one another, but can reflect recent dietary intakes and fluctuations in metabolic activities such as inflammation. Although plasma is often used as a general indication of selenium status, these difficulties can make interpretation difficult. Whole blood selenium concentrations are normally 10%–25% higher than plasma or serum concentrations, but plasma and serum concentrations are nonlinearly related to whole blood or red cells, correlating better at low levels of intake, but less accurately at higher levels of dietary intake. Thus whole blood selenium concentration is a more reflective measure of selenium status, especially at moderate to abundant nutritional intakes. Figure 7 shows the range of whole blood selenium concentrations observed in various countries around the world (27–35). As is apparent by the heterogeneity of blood selenium contents in various parts of North America, pronounced differences can be observed between continental regions. Selenium availability from food sources varies across the globe, in some cases resulting in dietary selenium status that is suboptimal (see Figure 7). The dotted line at 120 ng Se/mL indicates the whole blood selenium concentration required to attain optimal activities of selenium-dependent enzymes.

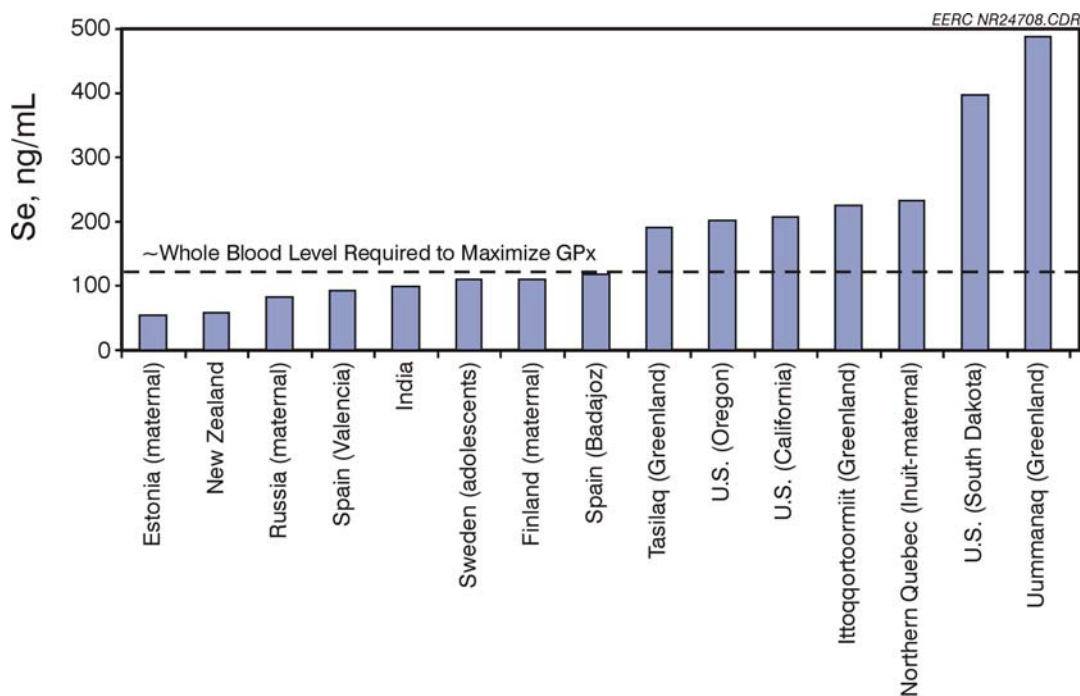


Figure 7. Global whole blood selenium concentrations.

Over 200 maternal and fetal whole blood samples from the current Seychelles Children’s Health and Development study were assessed for whole blood selenium. Of the total sample set, 148 paired sets of blood samples collected from mother and umbilical cord were collected at time of delivery. The blood selenium concentrations observed in the total sample set and from the subset of matched pairs of fetal and maternal samples are shown below:

<u>Cord blood $\mu\text{g/L}$</u>	<u>Maternal blood $\mu\text{g/L}$</u>	
Total sample set	134.7 \pm 48.7	182.0 \pm 42.3
(n)	(218)	(202)
Matched sample set	139.1 \pm 49.6	179.9 \pm 41.8
(n)	(148)	(148)

Our results indicate that maternal whole blood selenium concentrations were higher than fetal (cord blood) selenium. Observing maternal/fetal selenium data sets from a series of studies (1–3) in comparison to our results (Figure 8) reveals maternal transport of Se to the fetus is a tightly regulated process. The optimum whole blood selenium level to maximize GPx activity is generally accepted to be ~ 120 ng Se/mL. The accompanying graph suggests that in countries where maternal blood selenium was below this level, selenium was preferentially directed to the fetus. When maternal blood selenium was higher than this level, cord blood levels appeared to be homeostatically regulated towards this optimum.

The Faroes study did not examine maternal blood selenium levels and did not report mean and standard deviation data. The Faroes data reflected in the graph above represent the averaged mean of the multiple medians in the fetal whole blood selenium data sets and are presented here for approximate comparison to results from the Seychelles study.

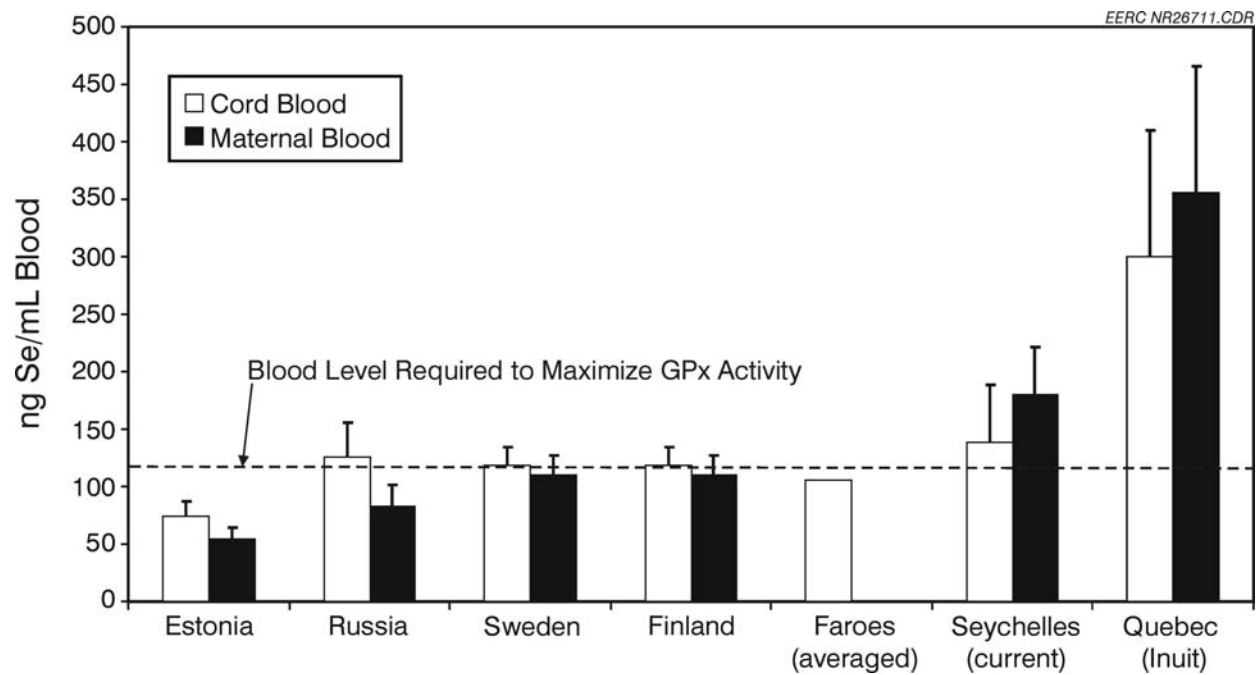


Figure 8. Comparisons of selenium in maternal and fetal whole blood samples collected at delivery.

A recent study examined mercury and selenium in hair samples collected from a fish-eating population of women that were either pregnant or nonpregnant age-matched controls (36). Although hair mercury levels in both groups were equivalent, hair selenium in pregnant women was $\sim\frac{1}{4}$ the concentration of hair selenium in age-matched controls. Low concentrations in hair of pregnant women indicates selenium diversion across the placenta to fulfill mineral requirements of the growing fetus. This may relate to homeostatic regulatory mechanisms in the placenta that maintain selenium at optimal levels as indicated in Figures 8 and 9.

Arranging maternal whole blood selenium concentrations in the 148 matched pairs in order of ascending concentration and segregating the data into ranked quartiles of 37 pairs per group, the ranked sets of maternal and fetal data are shown in Figure 9. As is apparent in the graph, cord blood selenium concentrations show signs of homeostatic regulation toward attaining the 120 ng Se/mL optimum.

The whole blood selenium data obtained in this study will be used in the multivariate analysis of fish consumption from food history, blood mercury, and neurofunctional end points. Effects of mercury exposure and the protective effects of dietary selenium will be assessed.

Pathology Study: Hg, Sb, and Se in Heart Tissues of Dilated Cardiomyopathy Patients

The Mayo Clinic Transplant Center conducts 20–25 heart transplants per year, about 10–12 of which are for patients with DCM. Samples of the explanted heart tissues removed from the patients receiving transplanted hearts are routinely preserved in formalin in blocks of paraffin for pathology studies and are stored in tissue repositories; however, such tissues are not appropriate for trace element or enzyme analysis. Frozen tissue samples collected from hearts of patients with DCM (and control hearts at autopsy) are now being collected for elemental analysis. These samples will be delivered to the University

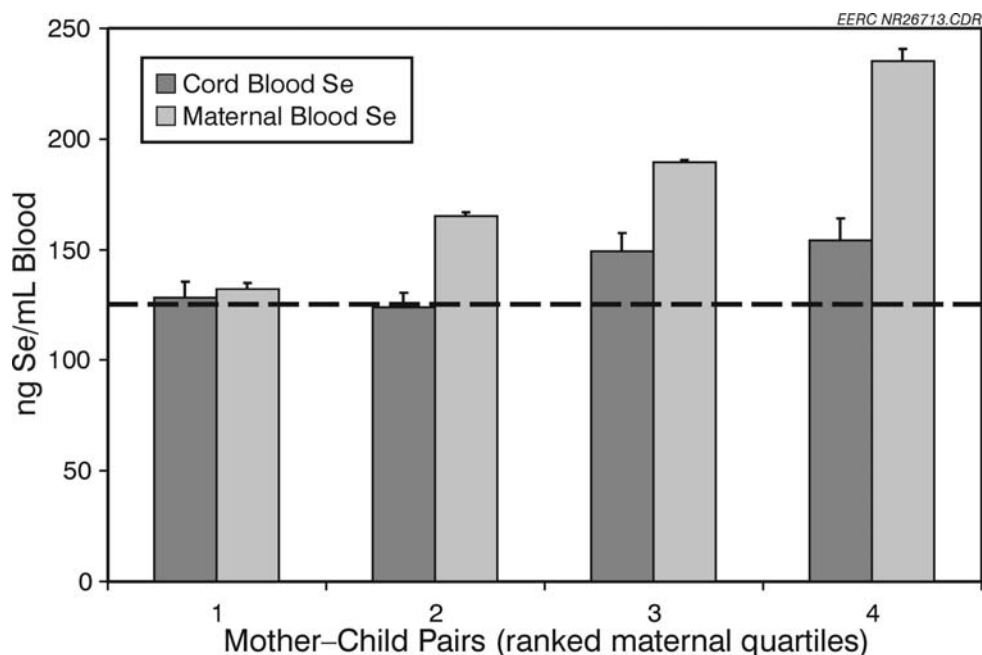


Figure 9. Selenium in ranked sets of blood samples.

of Missouri Research Reactor (MURR) for neutron activation analysis of their mercury, antimony, and selenium concentrations. Statistical analysis will be applied to determine whether heart tissues from DCM patients contain significantly different quantities of trace elements than normal control heart tissues.

Quality Assurance/Quality Control

Quality Objectives

The quality objectives of this project were to obtain statistically valid and physiologically meaningful results regarding the interactions of mercury and selenium. In the in vitro study, the quality objective was to obtain meaningful analytical data regarding mercury–selenium molecular species. In the dietary treatment study performed on experimental animals, being able to measure, contrast, and compare the weight gains of rats fed diets that varied in mercury and selenium contents provides a measure of their concentration-dependent effects. In the analysis of the Seychelles blood samples, the quality objective was to obtain analytically accurate and precise blood selenium determinations. In the study of elemental distributions in human heart samples, the quality objective was to obtain analytically accurate and precise determinations of elemental contents.

Measurement /Data Acquisition

XAFS spectra of mercury and selenium species occurring in whale liver samples were compared to authentic molecular forms used as analytical standards. Multiple spectra were collected from each sample, and mercury and selenium form analysis independently confirms HgSe occurrence and distribution in the whale liver tissues. Rat weights in the in vivo study were measured using calibrated instruments certified accurate to 0.1 g that were carefully checked using a weight standard at the start and finish of daily measurements. Rat weights were measured weekly, and observed values for each animal were individually plotted to validate consistency. All data points for each treatment group were included in determining the mean values and standard deviations for the weights at each weighing. Mercury and selenium contents in the hair, blood, and tissue samples were digested and analyzed alongside certified reference materials and calibration standards following standard protocols. Selenium contents in the Seychelles blood samples were each determined in triplicate alongside certified human blood quality control samples and calibration standards according to established protocols. Measurements of elemental compositions of hearts from normal hearts removed at autopsy are being compared to hearts from patients with DCM alongside certified quality control samples and calibration standards according to established protocols.

Assessment and Validation

The analysis data from the standard protocols used in this project indicate acceptable analytical accuracy and precision. Certified reference materials and control sample analytical results were within the expected range. The analytical results from the animal study reflect expected trends in measured indices. The heart tissue analysis task currently under way will be performed according to standard protocols, and results will be validated using certified reference materials and control samples.

Potential Users/Technology Transfer

The findings of these studies will provide important information for the U.S. Environmental Protection Agency, the U.S. Department of Energy, the U.S. Food and Drug Administration, and the World Health Organization. This information may assist these agencies in making regulatory policy

decisions regarding mercury exposure. Recognizing the influence of molar relationships between mercury and selenium will help these agencies in assessing the risks of human mercury exposure and identifying populations at risk.

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