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Factors Affecting the Bioaccessibility of Methylmercury in Several Marine Fish Species

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ABSTRACT: Bioaccessibility refers to the maximum bioavailability of pollutant ingested with food, and its measurements can lead to a more accurate risk assessment as compared to the measurements of total concentrations of pollutant in food. This study examined the factors affecting the bioaccessibility of methylmercury (MeHg) in nine species of marine fish with an aim to identify ways of reducing MeHg bioaccessibility. MeHg bioaccessibility without any treatment in the nine species of marine fish ranged from 16.0 to 67.7%. Steaming, grilling, and frying reduced MeHg bioaccessibility by 29.4−77.4% for rabbitfish and 74.6−95.8% for grouper. Co-consumption of phytochemical-rich foods such as green tea decreased the bioaccessibility of MeHg by 72.2% for rabbitfish and 74.0% for grouper, whereas *meso*-2,3-dimercaptosuccinic acid increased it by 39.2−108% for rabbitfish and 45.3−75.7% for grouper. The bioaccessibilities of both MeHg and inorganic mercury were independent of the total Hg concentration and the exposure route (dietary vs dissolved). In eight of the nine species studied, bioaccessibility was negatively correlated with the extent to which MeHg was partitioned into the metal-rich granule fraction and the trophically available fraction. It was positively correlated with partitioning into the cellular debris fraction. This study demonstrated the important control of subcellular distribution in MeHg bioaccessibility.

KEYWORDS: methylmercury, fish, bioaccessibility, subcellular distribution

INTRODUCTION

Fish are highly nutritious food rich in proteins, unsaturated lipids, minerals, vitamins, and nucleic acid.[1,2] With the rapid development of fish aquaculture, fish consumption has been increasing steadily. However, fish also accumulate micropollutants, such as toxic metals, and can become a significant source of such pollutants for humans. Mercury is recognized as one of the most toxic and hazardous pollutants and has caused worldwide concern due to its high toxicity, mobility, and bioaccumulation. Mercury exhibits various chemical forms with quite different toxicities in fish.[3,4] Methylmercury (MeHg) is well-known for its high neurotoxicity, easy penetration across biological membranes, efficient bioaccumulation, high stability, and long-term retention in biological tissues.[5−7] It is the major chemical form of mercury stored in fish muscle tissues (80−90% of the total mercury).[3−6]

Total concentration of pollutants and fish consumption rate are the traditional indices used in assessing the health risks of fish consumption, but the total concentration of pollutants ingested with food may not always reflect the real oral bioavailability of pollutants. When a pollutant is ingested with food, it is first released from the food matrix, is absorbed across the intestinal epithelium, and then exerts its toxic effects.[8,9] Bioaccessibility is generally defined as the fraction of pollutant ingested with food that is released from the food matrix into the digestive fluids and then potentially absorbed by the intestine. As such, it refers to the maximum bioavailability of the pollutant ingested with food.[8−12] Both in vivo and in vitro methods are now commonly used to evaluate bioaccessibility.[8−12] The in vivo methods are inherently more convincing, but expensive and laborious. The in vitro methods are rapid, simple, economical and have high reproducibility.[6,13,14] Several in vitro digestion models have now been developed to determine bioaccessibility and are becoming increasingly important in risk assessment.[8] Presently, bioaccessibility is an operationally defined term with respect to the in vitro digestion scheme used.

Several previous studies have examined the bioaccessibility of mercury in fish. Cabanero et al.[5,6] found that mercury bioaccessibility in fish was correlated with the selenium content and the Hg/Se ratio. Torres-Escribano et al.[15] recently showed that Hg bioaccessibility depended on its speciation, with 94% of the bioaccessible total mercury in swordfish being methylmercury. However, Cabanero et al.[5] showed that MeHg bioaccessibility was much lower than that of inorganic mercury in some fish. Co-consumption of fish with phytochemical-rich foods (e.g., tea, soy protein, and wheat bran) may significantly reduce mercury bioaccessibility.[16] Mercury bioaccessibility in fish seems to be independent of mercury concentration.[17]

Recent studies have shown that the subcellular distribution of metals can provide valuable information about metal toxicity and tolerance, as well as trophic transfer and bioaccumulation.[18−21] An earlier study in our laboratory has shown a significant relationship between bioaccessibility and the subcellular distribution of some trace elements (As, Cd, Cu, Fe, Se. and Zn) in two farmed marine fish.[22] For Hg, however, there is a general lack of such information, as well as information on the factors affecting mercury bioaccessibility. A few studies have shown that

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heating can affect the bioaccessibility of metals,[10,22−24] but whether or not cooking has some impact on methylmercury bioaccessibility remains unknown. *meso*-2,3-Dimercaptosuccinic acid (DMSA) is an efficient chelating agent for mercury usually used for treating mercury poisoning,[25] but information about the effects of DMSA on methylmercury bioaccessibility is also lacking.

This study was designed to determine the important factors affecting MeHg bioaccessibility in marine fish, hoping to find objective ways to reduce the potential risk of MeHg associated with fish consumption. The influences of DMSA, different cooking processes, and several phytochemical-rich foods on MeHg bioaccessibility in rabbitfish and grouper were explored. The influences of total concentration, exposure route, and mercury speciation on mercury bioaccessibility were also assessed using radiotracer methods. The subcellular distribution of MeHg in eight marine fish species was quantified for the first time, and its relationship with MeHg bioaccessibility was determined. To further confirm the importance of subcellular fractionation, the Hg from different subcellular fractions was purified and the bioaccessibility of Hg from each subcellular fraction was determined directly. Such study can provide a mechanistic understanding of subcellular distribution in controlling Hg bioaccessibility from marine fish.

MATERIALS AND METHODS

Samples and Reagents. The test subjects were live rabbitfish (*Siganus oramin*, 18.1 ± 0.72 cm in length and weighing 99.0 ± 12.5 g), grouper (*Epinephelus coioides*, 31.1 ± 1.36 cm and 429 ± 27.6 g), mullet (*Mugil cephalus*, 31.4 ± 0.81 cm and 227 ± 27.0 g), sillago (*Sillago japonica*, 20.7 ± 0.41 cm and 69.4 ± 3.70 g), yellow croaker (*Larimichthys crocea*, 21.2 ± 0.69 cm and 102 ± 10.4 g), golden thread (*Nemipterus virgatus*, 25.9 ± 1.13 cm and 107 ± 5.78 g), horsehead (*Branchiostegus argentatus*, 21.0 ± 1.08 cm and 86.7 ± 11.0 g), and mackerel (*Rastrelliger faughni*, 21.2 ± 0.54 cm and 58.9 ± 8.30 g), all purchased from a local market in Hong Kong. Black seabream (*Sparus macrocephalus*, 9.88 ± 0.57 cm and 18.4 ± 4.14 g) were purchased from a local Hong Kong fish farm. Among these fish species, mackerel and mullet are omnivores feeding on detritus and copepods; rabbitfish are herbivorous and feed primarily on benthic algae; the grouper, sillago, yellow croaker, golden thread, and horsehead are carnivorous and feed on other fish, octopus, crab, and shellfish (http://www.hk-fish.net). The black seabream is also omnivorous, feeding on shellfish, polychaetes, fish, and algae. The edible muscle tissues were removed from the fish and stored at −80 °C.

The chemicals for in vitro digestion (α-amylase, uric acid, mucin, bovine serum albumin, pepsin, pancreatin, lipase, bile, urea, glucose, glucuronic acid, glucosamine hydrochloride, KCl, and $NaHCO_3$) were all purchased from Sigma-Aldrich, Hong Kong. Tuna fish flesh homogenate IAEA436 (3.67 μg MeHg/g homogenate, from International Atomic Energy Agency, Vienna, Austria) was used as a standard reference material for MeHg determination. Green tea extract (GNC brand, containing 14% polyphenols), oranges, mangos, and cucumbers were purchased from a local supermarket in Hong Kong. The oranges, mangos, and cucumbers were squeezed and their juices used for the experiments. DMSA was also purchased from Sigma-Aldrich. All other reagents were of ultrapure or analytical grade, and the purified water was of Milli-Q grade.

Total MeHg Determination and Its Subcellular Distribution. The total concentration and subcellular distribution of MeHg in the fish muscle were determined. The tissues were first digested in 25% KOH methanol, and the total MeHg concentrations were measured using cold vapor atomic fluorescence spectrometry (CV-AFS, from Brooks Rand Laboratories, Seattle, WA) following distillation, aqueous ethylation, and purge and trap procedures as specified in EPA method 1630. Tuna fish flesh homogenate IAEA436 was concurrently digested and its MeHg concentration quantified. MeHg subcellular distribution was determined using the method described by Wallace.[18−20] A total of five fractions were obtained, including metal-rich granules (MRG), organelles, cellular debris, heat-stable protein (HSP), and heat-denaturable protein (HDP). These five fractions were digested with 25% KOH methanol solution and analyzed for their individual MeHg concentrations using CV-AFS. In the radiotracer experiments, the total concentrations and subcellular distributions of MeHg were determined directly using a Wallac 1480 NaI(T1) gamma counter (from Wallac, Turku, Finland).

Hg Bioaccessibility Determination by an in Vitro Digestion Method. Fish muscle samples were first minced and homogenized and then subjected to an in vitro digestion model as described by Versantvoort,[12] which simulated the three digestion processes in the human mouth, stomach, and intestines. Briefly, 4.5 g of each fish muscle homogenate was first incubated with 6 mL of artificial saliva for 5 min, then with 12 mL of artificial gastric juice for 2 h, and finally with a mixture of 12 mL of artificial duodenal juice, 6 mL of artificial bile, and 2 mL of 1 M HCO_3^- for another 2 h. All incubations were conducted in a shaker in 55 rpm at 37 °C. The supernatants and pellets were obtained by centrifugation at 2008*g* for 5 min. The MeHg contents in the supernatants were analyzed either by CV-AFS in stable experiments or by direct gamma counting in radiotracer experiments. Mercury bioaccessibility was calculated as the mercury in the supernatant as the percentage of total mercury in the 4.5 g of fish muscle. Generally, five fish tissue replicates were measured in each experiment.

Bioaccessibility Determination by Radiolabeling Methodology. A radiolabeling methodology was used to assess the impacts of different exposure routes, total Hg concentration, and mercury speciation on mercury bioaccessibility, using black seabream as the experimental fish. The radioisotopes $Me^{203}Hg$ and $^{203}Hg(II)$ were used as radiotracers. The live fish were exposed to two different concentrations of spiked Hg(II) [at either 5 ng/L or 5 μg/L, including both the isotopic spike and stable Hg(II)] or MeHg (at either 1 or 100 ng/L, including both isotopic spike and stable MeHg). The exposure lasted 7 days, during which the exposure concentrations were maintained relatively constant by renewing the radiotracers in the seawater every 2 days as well as by adding the appropriate radiotracer as required. In another treatment (dietary exposure), $Me^{203}Hg$ and $^{203}Hg(II)$ were first added to artificial fish feed at a concentration of 106 ng/g for Hg(II) or 48.7 ng/g for MeHg. The radiolabeled feed was then fed to the fish for 7 days. After the exposure, the fish muscles were processed and the bioaccessibilities of both MeHg and Hg(II) quantified as described above. The radioactivity of ^{203}Hg was measured by Wallac 1480 NaI(T1) gamma counter.

Effects of Cooking, DMSA, or Phytochemical-Rich Foods on MeHg Bioaccessibility. An in vitro digestion model was used to assess the effects of cooking (steaming, grilling, and frying) and of phytochemical-rich foods (green tea, orange, mango, and cucumber juices) on the bioaccessibility of MeHg in rabbitfish and grouper. The effect of DMSA was also determined using these species. The total MeHg concentrations in the rabbitfish and grouper muscle were first measured. For steaming, the fish muscle was steamed for 5 min in a glass Petri dish (14 cm in diameter). For frying, the fish muscle was fried for 5 min in 20 mL of canola oil (Imperial Banquet brand, 100% pure) in a glass Petri dish (14 cm in diameter). During grilling, no oil was added, and the fish muscle was grilled for 25 min in an oven. Then 4.5 g of cooked sample was digested in vitro as previously described for bioaccessibility determination. To test the influence of phytochemical-rich foods on MeHg bioaccessibility, either 315 mg of green tea extract power

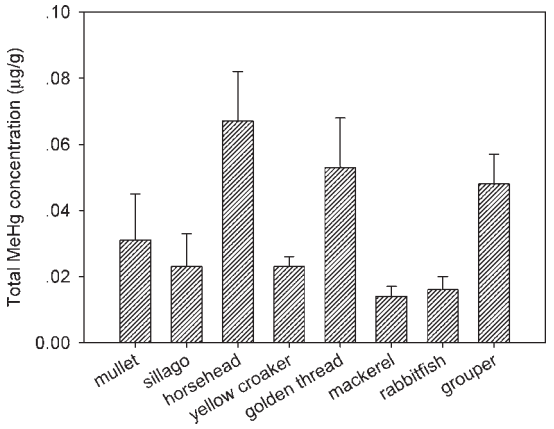

Figure 1. Total concentration of MeHg (μg/g wet weight) in the muscle of the eight marine fish species. Data are the mean ± SD (n = 5).

or 200 μL of a fruit juice was added to the fish before digestion. For the DMSA experiments, the amount of DMSA to be added in the in vitro digestion was calculated according to the total MeHg concentration in the rabbitfish and grouper muscles. Molar DMSA to MeHg ratios of 1:1, 10:1, and 50:1 were tested, with no DMSA addition as the control. The DMSA was first dissolved in distilled water.

Bioaccessibilities from Different Subcellular Fractions. The bioaccessibilities of MeHg and Hg(II) from different purified subcellular fractions (MRG, organelles, cellular debris, HSP, and HDP) were quantified. With rabbitfish and grouper, the muscle tissues were removed and subjected to subcellular fractionation as described in Wallace.[18−20] The bioaccessibility of each fraction was then determined. For black seabream, the radiolabeled fish were dissected and the muscle tissues were fractionated into different subcellular fractions. The bioaccessibility of the MeHg and Hg(II) in each fraction was then measured. In this experiment, the radioactivity was measured using the gamma counter.

Data Analysis. SPSS 10.0 software was applied in the statistical analysis. Statistical differences between the treatments were analyzed by a one-way analysis of variance (ANOVA) and Tukey tests. A significance level of $p < 0.05$ was adopted for all comparisons.

RESULTS AND DISCUSSION

Total Concentration, Exposure Route, and Speciation of Mercury and Its Bioaccessibility. The total concentration of MeHg in the eight marine fish species varied from 0.014 to 0.067 μg/g wet weight (Figure 1). These concentrations were below the recommended level of 0.30 μg/g ww in fish tissue by the U.S. Environmental Protection Agency (U.S. EPA, 2001),[26,27] suggesting that the fish were safe for human consumption and suitable for the bioaccessibility determination. There was no correlation between MeHg bioaccessibility and its total concentration in the eight marine fish species ($p > 0.05$, $r^2 = 0.326$). On the other hand, the radioactive experiment also examined whether different accumulated concentrations of mercury affected the bioaccessibility of Hg(II) and/or MeHg in black seabream. For both Hg species, there was no significant difference in their bioaccessibility [61−62% for Hg(II) and 68% for MeHg] between the two concentration treatments following 7 days of dissolved exposure (Table 1). An earlier study similarly showed that mercury bioaccessibility from some traditional foods was independent of mercury concentration.[17] Thus, other factors instead of total concentration may affect the MeHg bioaccessibility. As a result, MeHg bioaccessibility may be more accurate than total MeHg concentration in improving the risk assessment of MeHg.

Table 1. Total Concentration and Bioaccessibility in Fish Muscle Tissue of Different Mercury Speciation Resulting from Different Exposure Routes (L, Low Concentration; H, High Concentration)[a]

	exposure concn	concn in muscle (ng/g)	bioaccessibility (%)
dissolved exposure			
Hg(II)-L	5 ng/L	0.05 ± 0.01 b	62.4 ± 2.56 a
Hg(II)-H	5 μg/L	5.4 ± 0.3 c	60.7 ± 1.65 a
MeHg-L	1 ng/L	0.06 ± 0.01 b	67.7 ± 4.78 b
MeHg-H	100 ng/L	10.1 ± 0.2 d	67.5 ± 1.03 b
dietary exposure			
Hg(II)	106 ng/g	0.008 ± 0.003 a	54.2 ± 5.71 a
MeHg	48.7 ng/g	9.2 ± 0.9 d	65.6 ± 1.05 b

[a] Different letters indicate that the different exposure groups were significantly different.

The radioactive experiments examined whether different exposure routes affected the bioaccessibility of Hg(II) and/or MeHg in black seabream. There was no significant difference in the Hg bioaccessibility between dissolved and dietary exposure (Table 1). For each exposure route, the bioaccessibility of MeHg was slightly higher than that of Hg(II). The difference may result from their different subcellular partitionings (see below).

Factors Affecting MeHg Bioaccessibility in Rabbitfish and Grouper. The baseline (without any treatment) MeHg bioaccessibility in rabbitfish was 26.5% and in grouper was 63.9%, but these decreased as a result of steaming, grilling, or frying (Figure 2a). Cooking appeared to have a greater effect on the bioaccessibility from grouper than from rabbitfish. MeHg bioaccessibility decreased by 74.6−95.8% for grouper but by only 29.4−77.4% for rabbitfish. Steaming was less effective than grilling, which was less effective than frying, in reducing bioaccessibility.

Foods are usually cooked before consumption. Our results indicate that cooking significantly reduces the MeHg bioaccessibility, and different cooking methods have different effects. These decreases in MeHg bioaccessibility as a result of cooking may be due to two reasons. The loss of water and weight during heating resulted in a loss of some labile proteins and, thus, reduced the metal bioaccessibility.[10] At the same time, denaturing the proteins of the muscle fibers led to tissue shrinkage and the formation of insoluble fractions, which made the proteins less digestible and their components less bioaccessible.[28] Previous studies have reported that cooking played different roles in the bioaccessibility of different metals.[22]

Figure 2b shows the bioaccessibility of the MeHg in rabbitfish and grouper tissue following additions of green tea extract or fruit juice (orange juice, mango juice, or cucumber juice). Green tea extract substantially decreased the MeHg bioaccessibility in both fish (by 72.2% for rabbitfish and 74.0% for grouper), but none of the juices had any significant effect on MeHg bioaccessibility from either fish.

Green tea, fruit juices, and other phytochemical-rich foods are often consumed with fish in the same meal. A previous study has shown that some phytochemical-rich foods such as green tea extract, black tea extract, and soy protein significantly reduce

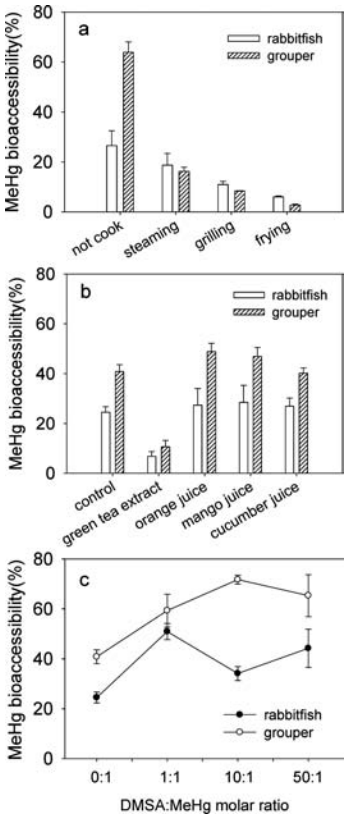

Figure 2. Bioaccessibility of MeHg in rabbitfish and grouper under different conditions: (a) bioaccessibility under different cooking methods; (b) bioaccessibility with addition of different phytochemical-rich foods; (c) bioaccessibility under different DMSA/MeHg ratios. Data are the mean ± SD (n = 5).

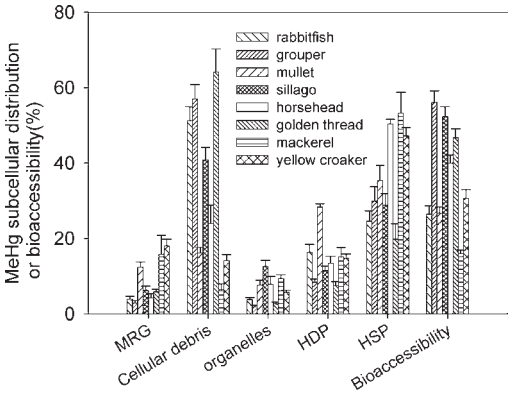

Figure 3. Subcellular distribution of MeHg in the eight marine fish species and bioaccessibility of MeHg from these fish species (last column). Data are the mean ± SD (n = 5).

mercury bioaccessibility,[16] although none has previously considered MeHg bioaccessibility. In this study, only the green tea extract significantly reduced MeHg bioaccessibility whereas the fruit juices were not, which was consistent with the finding of previous research.[16] Co-consumption of fish with green tea may significantly decrease the health risks of MeHg in fish. Green tea extract has abundant catechins, theaflavins, and flavonoids, and these have been shown to be good natural chelators and scavengers of metals.[29−34] Accordingly, the reduction of MeHg bioaccessibility resulting from green tea extract might be attributed to the constituents in green tea extract such as polyphenols and dietary fibers that could bind with MeHg in foods during human digestion,[16] which might enhance the excretion of MeHg through the formation of insoluble complexes, therefore reducing MeHg bioaccessibility. On the other hand, the green tea was added into the in vitro model as powder. It was possible that MeHg was bound with the powder form and remained in the precipitated phase, which might lead to a reduction of MeHg bioaccessibility.

DMSA is another efficient chelating agent for mercury. The effects of the different DMSA treatments are summarized in Figure 2c. The unexpected finding was that DMSA significantly raised the bioaccessibility of MeHg in both rabbitfish (by 39.2−108%) and grouper (by 45.3−75.7%) tissue. This result to some extent contradicts those reported by Shim et al.,[16] who showed that 2,3-dimercapto-1-propane sulfonate (DMPS, with a structure similar to that of DMSA) could significantly reduce mercury bioaccessibility from fish tissues in a dose-dependent manner. Generally, metal chelators can bind with metals and form insoluble complexes during human digestion, reducing the metals' bioaccessibility.[16,31,32,35,36] The increased MeHg bioaccessibility with DMSA in this study may have resulted from the way the DMSA was added. Shim et al.[16] added DMPS as a powder in in vitro digestion, and mercury was bound with DMPS in the precipitated phase, decreasing its bioaccessibility. In this study DMSA solution was used, and any MeHg bound with DMSA was left in the supernatant, where it might lead to an increase of MeHg bioaccessibility.

MeHg Subcellular Distribution and Its Bioaccessibility. Cellular debris and HSP were the primary pools for MeHg in all eight fish species tested (Figure 3). Only a small percentage of MeHg was distributed in the MRG, organelles, and HDP, although the different species exhibited some differences in their subcellular distributions. For example, the percentage of MeHg distributed in cellular debris was much higher (40−64%) for rabbitfish, grouper, sillago, and golden thread than for mullet, horsehead, mackerel, and yellow croaker (6−24%).

Many previous studies have examined MeHg binding in fish tissues. MeHg has generally been found to bind to sulfhydryl-rich amino acids in fish and other seafoods.[37] For example, Harris et al.[4] found that MeHg in fish was bound with thiols, and Lemes and Wang[38] pointed out it was bound with cysteine. Binding with cysteine makes MeHg more bioavailable for transport across the blood−brain barrier,[38] and binding with peptides such as glutathione and metallothioneins (also named HSP in the subcellular distribution) or with elements such as selenium is considered as a detoxification strategy.[5,6,39,40] HSP (metallothioneins) could be induced by MeHg,[41,42] which may explain the large fraction of MeHg in HSP in the eight fish species. A earlier study also found that MeHg was mostly bound in HSP in the grunt *Terapon jarbua*.[39]

Our study presents the first systematic measurement of the subcellular distribution of MeHg in different marine fish. Among the species tested, mullet and mackerel are omnivorous fish, and their MeHg was mainly stored in HSP in their muscles. For the herbivorous rabbitfish and carnivores such as sillago, golden thread, and grouper, MeHg was predominantly stored in cellular debris. For the other carnivores (the horsehead and yellow croaker), HSP was still the dominant pool for MeHg. It is difficult to

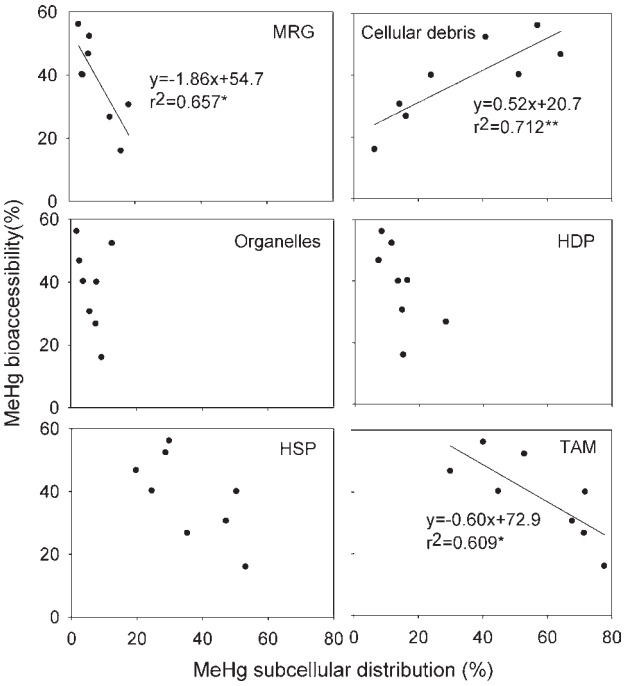

Figure 4. Relationship between MeHg bioaccessibility from the muscles of the eight fish species and the MeHg subcellular distribution. MRG, metal-rich granule; HDP, heat-denaturable protein; HSP, heat-stable protein; TAM, trophically available metal, organelles + HDP + HSP. *, $p < 0.05$; **, $p < 0.01$.

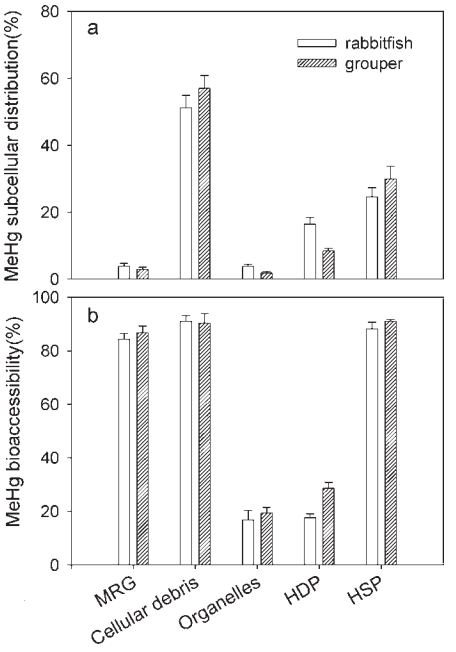

Figure 5. MeHg subcellular distribution (a) and bioaccessibility from each of the purified subcellular fractions (b) in rabbitfish and grouper. Data are the mean ± SD ($n = 5$).

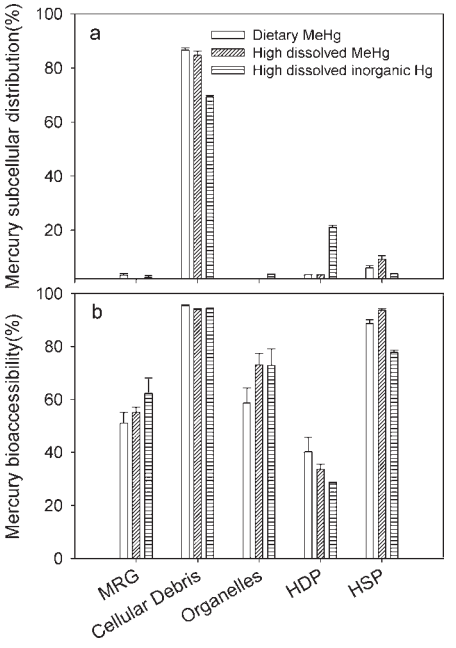

Figure 6. Subcellular distributions of MeHg and Hg(II) (a) and their bioaccessibilities from each of the purified subcellular fractions (b) in black seabream following radioactive exposure to aqueous or dietary mercury. Data are the mean ± SD ($n = 5$).

conclude that the subcellular distribution was dependent on the feeding habits of the fish. However, different prey of fish might have different subcellular fractionation of MeHg. Cheung et al.[43] have demonstrated that subcellular metal distributions in predators could be affected by metal fractionation in their prey, so different MeHg fractionations in prey may have led to different distributions in fish observed here.

MeHg bioaccessibility varied with fish species, ranging from 16 to 56% (Figure 3). Bioaccessibility was highest in the grouper (56%), whereas that of mackerel was the lowest (16%). In general, the bioaccessibility from herbivorous and omnivorous fish (mullet, rabbitfish, and mackerel) was low, whereas it was somewhat higher from the carnivores (yellow croaker, horsehead, sillago, grouper, and golden thread). Their different feeding habits may lead to different accumulation patterns, subcellular distributions, and hence bioaccessibilities. Recently, Torres-Escribano et al.[15] have quantified the bioaccessibility of total mercury (64%) in swordfish and found that 94% of the bioaccessible mercury was MeHg, suggesting that this carnivorous fish also has a high MeHg bioaccessibility.

Correlation analysis was conducted between subcellular distribution and bioaccessibility of MeHg in the eight fish species (Figure 4). There was a significant negative correlation between MeHg bioaccessibility and the fraction distributed in the MRG and TAM ($p < 0.05$). There was also a significant positive correlation between bioaccessibility and the fraction found in the cellular debris ($p < 0.01$). For the other subcellular fractions, no significant correlation was apparent.

MRG is generally regarded as a less bioavailable fraction,[19] and previous work in our laboratory has also suggested that metals bound with MRG have low bioaccessibility.[22] Nevertheless, MeHg bioaccessibility from purified MRG was found in this study to be high (e.g., ~80% in rabbitfish and grouper, Figure 5b). Thus, the negative correlation of MeHg bioaccessibility with MeHg subcelluar distribution may have been due to the low partitioning of MeHg into the MRG (e.g., ~3% in rabbitfish and grouper, Figure 5a). In contrast, the positive correlation of MeHg bioaccessibility with MeHg partitioning into cellular debris may be due to high bioaccessibility from cellular debris as well as high

MeHg partitioning into those cellular debris (Figures 5 and 6). Such a significant correlation was in contrast to our previous findings with other trace elements including As, Cd, Cu, Fe, Se, and Zn, in which no correlation of element bioaccessibility and element partitioning into the cellular debris was observed.[22] The difference might be due to the different partitionings in the cellular debris fraction. For example, the partitioning of those trace elements in the fish cellular debris in our earlier study[22] was within a narrow range and was lower than the partitioning of MeHg observed in this study.

TAM is defined as a subcellular compartmentalization consisting of organelles, HDP, and HSP, which represented the soluble and the bioavailable metals from the diet.[20] A previous study in our laboratory[22] has demonstrated a significant positive correlation between an element's bioaccessibility and its distribution in the TAM fraction. It thus appears that a trace element's partitioning into this fraction may reflect the potential bioavailability to human consumers. Contrary to our expectation, we found a negative relationship between MeHg bioaccessibility and its partitioning in TAM in this study. The observed relationship was primarily driven by the MeHg distribution in the HSP, which was one of the dominant pools for MeHg in the eight fish species (20−53%) and was highly bioaccessible on the basis of the extraction of the purified HSP fraction (Figure 5b). There have been very few studies on the controls of subcellular distribution on mercury bioavailability. Earlier studies have demonstrated a significant relationship between the dietary assimilation efficiency (AE) of metals (Cd, Zn, and Se) in predators (fish and snails) and the distribution of those metals in the TAM fraction of the prey.[44,45] In a recent study, Dang and Wang[39] found no significant relationship between the AEs and the TAM fractions for Hg(II) or MeHg in the marine fish *Terapon jarbua*. The MeHg AEs were larger than its distribution in the TAM fraction, indicating that MeHg bound to other fractions (MRG and/or cellular debris) was also bioavailable. For Hg(II), a proportion categorized in the TAM fraction was not actually 100% trophically available. Thus, TAM is not an appropriate predictor for Hg(II) or MeHg dietary bioavailability in these fish.

Underlying Mechanisms of Mercury Subcellular Distribution Controlling Its Bioaccessibility. Bioaccessibility determination of purified subcellular fractions might provide a possible explanation for the underlying mechanisms of mercury subcellular distribution in controlling its bioaccessibility. Individual bioaccessibility measurements were performed with all five purified subcellular fractions from the rabbitfish, grouper, and black seabream. In rabbitfish and grouper (Figure 5), generally, the MeHg bioaccessibility from MRG, cellular debris, and HSP fractions were all high, ranging from 84 to 91%, whereas those from organelles and HDP were exceptionally low (17−29%). There was no significant difference between the two fish species. The radiolabeled fish black seabream were also first subjected to subcellular fractionation, and then each purified fraction was quantified for mercury bioaccessibility. In this experiment, three treatments (dietary exposure of MeHg and dissolved exposure with high concentrations of either Hg(II) and MeHg) were measured due to the relatively high radioactivity in the fish (Figure 6). Cellular debris was the predominant pool for both MeHg and Hg(II) in all three treatments, but there were still significant differences in the subcellular distribution into organelles, HDP, and HSP between MeHg and Hg(II). The bioaccessibilities from cellular debris and HSP were the highest, followed by organelles > MRG > HDP (Figure 6). The bioaccessibility resulting from dietary exposure was significantly different from that of dissolved exposure in both the organelles and HDP fractions. Both Hg(II) and MeHg had comparable bioaccessibilities from each subcellular fraction, except the HDP fraction.

The bioaccessibilities of purified subcellular fractions were different between the radioactive labeling (black seabream, Figure 6) and nonradioactive (rabbitfish and grouper, Figure 5) experiments. The difference was mainly in the MRG, organelles, and HDP. In radiolabeled experiments, the mercury bioaccessibility of purified MRG was lower, whereas those of organelles and HDP were higher, than in the nonradioactive experiments. Such differences may have been due to the different accumulation times, leading to different accumulation forms, or to the different species used in the experiments. Mercury was mainly distributed in cellular debris and HSP in the rabbitfish and grouper, but was mainly stored in cellular debris with little in HSP in black seabream. However, in all fish species, the bioaccessibilities of purified MRG and cellular debris were rather high. MRG and cellular debris was regarded as insoluble subcellular fractions.[18−21] The results indicate that the insoluble factions could also be highly bioaccessible and be assimilated by humans like the soluble TAM fraction. Dang and Wang[39] also indicated that MeHg bound with the insoluble fractions was bioavailable.

The overall Hg bioaccessibility can be calculated on the basis of the measured bioaccessibility of the five purified fractions and the distribution of Hg in each subcellular fraction. For rabbitfish and grouper (nonradioactive experiments), the overall mercury bioaccessibilities were 183.8 and 31.6% more than that measured by the in vitro digestion, respectively. In the radioactive experiments, the overall mercury bioaccessibility was 39.3, 34.8, and 29.0% more than that measured by the in vitro digestion for the dietary exposure of MeHg, dissolved exposure with high MeHg concentration, and dissolved exposure with high Hg(II) concentration treatments, respectively. These calculations suggest that the overall bioaccessibility calculated from the purified five fractions was higher than the actual bioaccessibility determined in vitro. The difference was probably caused by interaction among the five subcellular fractions. The subcellular fractions were processed by homogenization, high-speed centrifugation, and heat treatment of the muscle tissue. With a purified fraction, the interaction of the fractions might disappear and their structures might have unfolded, facilitating the release of Hg and thus increasing its bioaccessibility. For the unpurified tissues, the structure of the tissues might inhibit the action of digestive enzymes and the release of Hg.

Our results suggested that Hg bioaccessibility in fish muscles may be not only controlled by its distributions in the subcellular fractions and the bioaccessibility of individual subcelluar fractions but also controlled by the interaction of the subcellular fractions. Consequently, all subcellular fractions as well as their interaction may need to be considered for their controls of Hg bioaccessibility.

Conclusions. The total MeHg concentrations in eight marine fish species did not exceed the levels recommended by the U.S. EPA. There was no correlation between the bioaccessibility of MeHg and its total concentration, suggesting that bioaccessibility study was required to improve the risk assessment of MeHg. Mercury bioaccessibility depended on the fish species and their different feeding habits and also on cooking methods, other items consumed with the fish, and the mercury's subcellular distribution and speciation. The bioaccessibility of mercury in herbivorous and omnivorous fish was generally low, but it was higher in

carnivorous fish. Choosing herbivorous and omnivorous fish to consume may potentially diminish the MeHg health hazards for humans. Steaming, grilling, or frying greatly decreased MeHg bioaccessibility, as did co-consumption with green tea. Orange, mango, and cucumber juices did not impede bioaccessibility, and DMSA increased it. MeHg bioaccessibility in fish muscles was not only controlled by its subcellular distribution and the bioaccessibility of individual subcelluar fractions but was also controlled by the interaction of the different fractions. As a result, MeHg subcellular distribution was an important factor affecting its bioaccessibility, and it should be considered in any risk assessment.

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REFERENCES

(1) Verbeke, W.; Sioen, I.; Pieniak, Z.; Van Camp, J.; De Henauw, S. Consumer perception versus scientific evidence about health benefits and safety risks from fish consumption. *Public Health Nutr.* **2005**, *8*, 422–429.

(2) Domingo, J. L.; Bocio, A.; Falco, G.; Llobet, J. M. Benefits and risks of fish consumption. Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Toxicology* **2007**, *230*, 219–226.

(3) George, G. N.; Singh, S. P.; Prince, R. C.; Pickering, I. J. Chemical forms of mercury and selenium in fish following digestion with simulated gastric fluid. *Chem. Res. Toxicol.* **2008**, *21*, 2106–2110.

(4) Harris, H. H.; Pickering, I. J.; George, G. N. The chemical form of mercury in fish. *Science* **2003**, *301*, 1203.

(5) Cabanero, A. I.; Madrid, Y.; Camara, C. Mercury-selenium species ratio in representative fish samples and their bioaccessibility by an *in vitro* digestion method. *Biol. Trace Elem. Res.* **2007**, *119*, 195–211.

(6) Cabanero, A. I.; Madrid, Y.; Camara, C. Selenium and mercury bioaccessibility in fish samples: an *in vitro* digestion method. *Anal. Chim. Acta* **2004**, *526*, 51–61.

(7) Chapman, L.; Chan, H. M. The influence of nutrition on methyl mercury intoxication. *Environ. Health Perspect.* **2000**, *108*, 29–56.

(8) Oomen, A. G.; Hack, A.; Minekus, M.; Zeijdner, E.; Cornelis, C.; Schoeters, G.; Verstraete, W.; Van de Wiele, T.; Wragg, J.; Rompelberg, C. J.; Sips, A. J.; Van Wijnen, J. H. Comparison of five *in vitro* digestion models to study the bioaccessibility of soil contaminants. *Environ. Sci. Technol.* **2002**, *36*, 3326–3334.

(9) Oomen, A. G.; Rompelberg, C. J. M.; Bruil, M. A.; Dobbe, C. J. G.; Pereboom, D. P. K. H.; Sips, A. J. A. M. Development of an *in vitro* digestion model for estimating the bioaccessibility of soil contaminants. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 281–287.

(10) Amiard, J. C.; Amiard-Triquet, C.; Charbonnier, L.; Mesnil, A.; Rainbow, P. S.; Wang, W.-X. Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia. *Food Chem. Toxicol.* **2008**, *46*, 2010–2022.

(11) Brandon, E. F. A.; Oomen, A. G.; Rompelberg, C. J. M.; Versantvoort, C. H. M.; van Engelen, J. G. M.; Sips, A. J. A. M. Consumer product *in vitro* digestion model: bioaccessibility of contaminants and its application in risk assessment. *Regul. Toxicol. Pharmacol.* **2006**, *44*, 161–171.

(12) Versantvoort, C. H. M.; Oomen, A. G.; Van de Kamp, E.; Rompelberg, C. J. M.; Sips, A. J. A. M. Applicability of an *in vitro* digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* **2005**, *43*, 31–40.

(13) Laparra, J. M.; Glahn, R. P.; Miller, D. D. Bioaccessibility of phenols in common beans (*Phaseolus vulgaris* L.) and iron (Fe) availability to Caco-2 cells. *J. Agric. Food Chem.* **2008**, *56*, 10999–11005.

(14) Laparra, J. M.; Velez, D.; Montoro, R.; Barbera, R.; Farre, R. Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. *J. Agric. Food Chem.* **2003**, *51*, 6080–6085.

(15) Torres-Escribano, S.; Velez, D.; Montoro, R. Mercury and methylmercury bioaccessibility in swordfish. *Food Addit. Contam.* **2010**, *27*, 327–337.

(16) Shim, S. M.; Ferruzzi, M. G.; Kim, Y. C.; Janle, E. M.; Santerre, C. R. Impact of phytochemical-rich foods on bioaccessibility of mercury from fish. *Food Chem.* **2009**, *112*, 46–50.

(17) Laird, B. D.; Shade, C.; Gantner, N.; Chan, H. M.; Siciliano, S. D. Bioaccessibility of mercury from traditional northern country foods measured using an *in vitro* gastrointestinal model is independent of mercury concentration. *Sci. Total Environ.* **2009**, *407*, 6003–6008.

(18) Wallace, W. G.; Lee, B. G.; Luoma, S. N. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). *Mar. Ecol.: Prog. Ser.* **2003**, *249*, 183–197.

(19) Wallace, W. G.; Lopez, G. R.; Levinton, J. S. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. *Mar. Ecol.: Prog. Ser.* **1998**, *172*, 225–237.

(20) Wallace, W. G.; Luoma, S. N. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). *Mar. Ecol.: Prog. Ser.* **2003**, *257*, 125–137.

(21) Wang, W.-X.; Rainbow, P. S. Subcellular partitioning and the prediction of cadmium toxicity to aquatic organisms. *Environ. Chem.* **2006**, *3*, 395–399.

(22) He, M.; Ke, C. H.; Wang, W.-X. Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. *J. Agric. Food Chem.* **2010**, *58*, 3517–3523.

(23) Almela, C.; Laparra, J. M.; Velez, D.; Barbera, R.; Farre, R.; Montoro, R. Arsenosugars in raw and cooked edible seaweed: characterization and bioaccessibility. *J. Agric. Food Chem.* **2005**, *53*, 7344–7351.

(24) Hemalatha, S.; Platel, K.; Srinivasan, K. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *J. Trace Elem. Med Biol.* **2007**, *21*, 1–7.

(25) Chekmeneva, E.; Diaz-Cruz, J. M.; Arino, C.; Esteban, M. Study of the Hg^{2+} binding with chelation therapy agents by differential pulse voltammetry on rotating Au-disk electrode and electrospray ionization mass-spectrometry. *Anal. Chim. Acta* **2009**, *653*, 77–85.

(26) Hothem, R. L.; Jennings, M. R.; Crayon, J. J. Mercury contamination in three species of anuran amphibians from the Cache Creek Watershed, California, USA. *Environ. Monit. Assess.* **2010**, *163*, 433–448.

(27) Le, D. Q.; Nguyen, D. C.; Harino, H.; Kakutani, N.; Chino, N.; Arai, T. Distribution of trace metals and methylmercury in soft tissues of the freshwater eel *Anguilla marmorata* in Vietnam. *Arch. Environ. Contam. Toxicol.* **2010**, *59*, 282–290.

(28) Kulp, K. S.; Fortson, S. L.; Knize, M. G.; Felton, J. S. An *in vitro* model system to predict the bioaccessibility of heterocyclic amines from a cooked meat matrix. *Food Chem. Toxicol.* **2003**, *41*, 1701–1710.

(29) Chen, S. L.; Kim, E. K.; Shuler, M. L.; Wilson, D. B. Hg^{2+} removal by genetically engineered *Escherichia coli* in a hollow fiber bioreactor. *Biotechnol. Prog.* **1998**, *14*, 667–671.

(30) Graham, H. N. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* **1992**, *21*, 334–350.

(31) Hegde, M. L.; Bharathi, P.; Suram, A.; Venugopal, C.; Jagannathan, R.; Poddar, P.; Srinivas, P.; Sambamurti, K.; Rao, K. J.; Scancar, J.; Messori, L.; Zecca, L.; Zatta, P. Challenges

associated with metal chelation therapy in Alzheimer's disease. *J. Alzheimers Dis.* **2009**, *17*, 457–468.

(32) Mandel, S.; Amit, T.; Reznichenko, L.; Weinreb, O.; Youdim, M. B. H. Green tea catechins as brain-permeable, natural iron chelators-antioxidants for the treatment of neurodegenerative disorders. *Mol. Nutr. Food Res.* **2006**, *50*, 229–234.

(33) Ragan, M. A.; Smidsrod, O.; Larsen, B. Chelation of divalent metal ions by brown algal polyphenols. *Mar. Chem.* **1979**, *7*, 265–271.

(34) Record, I. R.; McInerney, J. K.; Dreosti, I. E. Black tea, green tea, and tea polyphenols. *Biol. Trace Elem. Res.* **1996**, *53*, 27–43.

(35) Beak, D. G.; Basta, N. T.; Scheckel, K. G.; Traina, S. J. Linking solid phase speciation of Pb sequestered to Birnessite to oral Pb bioaccessibility: implications for soil remediation. *Environ. Sci. Technol.* **2008**, *42*, 779–785.

(36) Subacz, J. L.; Barnett, M. O.; Jardine, P. M.; Stewart, M. A. Decreasing arsenic bioaccessibility/bioavailability in soils with iron amendments. *J. Environ. Sci. Health* **2007**, *42*, 1317–1329.

(37) Clarkson, T. W. The toxicology of mercury. *Crit. Rev. Clin. Lab. Sci.* **1997**, *34*, 369–403.

(38) Lemes, M.; Wang, F. Y. Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. *J. Anal. At. Spectrom.* **2009**, *24*, 663–668.

(39) Dang, F.; Wang, W.-X. Subcellular controls of mercury trophic transfer to a marine fish. *Aquat. Toxicol.* **2010**, *99*, 500–506.

(40) Bebianno, M. J.; Santos, C.; Canario, J.; Gouveia, N.; Sena-Carvalho, D.; Vale, C. Hg and metallothionein-like proteins in the black scabbardfish *Aphanopus carbo*. *Food Chem. Toxicol.* **2007**, *45*, 1443–1452.

(41) Nordberg, M. Metallothioneins: historical review and state of knowledge. *Talanta* **1998**, *46*, 243–254.

(42) Chan, J.; Huang, Z.; Merrifield, M.; Salgado, M.; Stillman, M. Studies of metal binding reactions in metallothioneins by spectroscopic, molecular biology, and molecular modeling techniques. *Coord. Chem. Rev.* **2002**, *233*−*234*, 319–339.

(43) Cheung, M. S.; Fok, E. M. W.; Ng, T. Y. T.; Yen, Y. F.; Wang, W.-X. Subcellular cadmium distribution, accumulation, and toxicity in a predatory gastropod, *Thais Clavigera*, fed different prey. *Environ. Toxicol. Chem.* **2006**, *25*, 174–181.

(44) Zhang, L.; Wang, W.-X. Significance of subcellular metal distribution in prey in influencing the trophic transfer of metals in a marine fish. *Limnol. Oceanogr.* **2006**, *51*, 2008–2017.

(45) Cheung, M. S.; Wang, W.-X. Influence of subcellular metal compartmentalization in different prey on the transfer of metals to a predatory gastropod. *Mar. Ecol.: Prog. Ser.* **2005**, *286*, 155–166.