Influence of Dietary Protein Levels on the Fate of Methylmercury and on Amino Acid Transport at the Renal Brush Border Membrane in Rats

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We investigated the influence of dietary protein levels on the fate of methylmercury (MeHg) in rats, and the difference in its urinary excretion was discussed from the viewpoint of the dietary protein level-dependent alteration in activity of the neutral amino acid transport system, through which MeHg metabolites such as MeHg-L-cysteine are reabsorbed, at the renal brush border membrane. When MeHg was administered to rats fed either a 24.8% protein diet (normal protein diet, NPD) or a 7.5% protein diet (low protein diet, LPD), urinary Hg excretion was much lower in LPD-fed rats than in NPD-fed rats, whereas no difference was observed in fecal excretion 1 day after MeHg administration. At that time, Hg concentrations in the brain and plasma were similar in the two dietary groups, whereas the concentrations in liver and blood were higher, but the renal concentration was lower in LPD-fed rats than in NPD-fed rats. Regardless of the presence of Na^+, uptake of ^{14}C-L-phenylalanine for the first 20 sec was higher in the renal brush border vehicles from LPD-fed rats than in those from NPD-fed rats. These results suggest that dietary protein deficiency enhances the neutral amino acid transport at the renal brush border membrane, which could lead efficient reabsorption of MeHg metabolites from the proximal luminal space, and it might play a key role in the marked decrease in urinary excretion of MeHg.

Key words —— dietary protein, methylmercury, amino acid transport, renal brush border membrane

INTRODUCTION

It is well known that methylmercury (MeHg), a major environmental pollutant, has a high affinity for the thiol group.[1,2] We earlier reported that the fate of MeHg was affected by various endogenous factors including glutathione (GSH)[3,4] and other thiol compounds.[5] Using mice, we have also demonstrated that dietary protein levels, which can modulate the GSH metabolism, are one of the important modifying factors determining the fate and toxicity of MeHg.[6–10] Markedly lower urinary Hg excretion and higher Hg levels in various tissues including the brain and kidney were observed in 7.5% protein diet (low protein diet, LPD)-fed mice than in 24.8% protein diet (normal protein diet, NPD)-fed mice after a single administration of MeHg.[6] We have suggested that one reason for the difference in the fate of MeHg between NPD- and LPD-fed mice is an alteration in the metabolism of thiol compounds such as GSH,[6] which is closely related to the fate of MeHg.[3,4] In addition, it also has been suggested that the more efficient transport activity of neutral amino acids into the brain in LPD-fed mice than in NPD-fed mice would contribute to the higher brain Hg concentration,[7,10,11] since MeHg is transported into the brain as its L-cysteine conjugate through the neutral amino acid transport system.[12–17] Furthermore, we showed that treatment with acivicin, a specific inhibitor of γ-glutamyltranspeptidase (γ-GTP),[18,19] markedly promoted urinary Hg excretion and resulted in similar excretion in the two dietary groups.[6] Since this suggested that the influx rates of low molecular weight (LMW) MeHg metabolites into the proximal luminal space, through glomerular filtration and through the secretion system for GSH and its S-conjugates,[4,20] would be similar in the two dietary groups,[6] we assumed that the marked decrease in urinary Hg excretion by the lowered dietary protein level might be caused by efficient re-

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absorption of MeHg metabolites such as MeHg-L-cysteine from the proximal luminal space.[6] However, there are few reports on the dietary protein level-dependent alterations in neutral amino acid transport at the renal brush border membrane, although it has been suggested that dietary adaptations in amino acid transport at the intestinal brush border membrane are distinct for essential and non-essential amino acids.[21]

In the present study, first, we examined tissue and excretory Hg in rats fed NPD or LPD after MeHg administration to determine whether the influence of dietary protein levels on the fate of MeHg would show a similar tendency in rats and mice. Then, uptake of a typical neutral amino acid across the renal brush border membrane obtained from NPD- or LPD-fed rats was investigated to account for the difference in urinary Hg excretion. The distribution of Hg in plasma was also determined, since plasma LMW MeHg metabolites, mainly MeHg-GSH and MeHg-L-cysteine, are a driving force in tissue uptake of MeHg including in the brain.[13,22]

MATERIALS AND METHODS

Reagents —— D-[^{14}C(U)]glucose and L-[^{14}C(U)]phenylalanine were purchased from Amarsham Biosciences Co. (Piscataway, NJ, U.S.A.). Methylmercuric chloride and L-γ-glutamyl-*p*-nitroanilide were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively. Other reagents used were reagent grade.

Animals —— Male Wistar rats (aged 7 weeks), obtained from CLEA Japan Co. (Osaka, Japan), were maintained at 23 ± 2°C and 50–60% relative humidity, and were exposed to a 12-hr light cycle from 7:00 a.m. The animals were acclimated to either of the two isocaloric powder diets, NPD and LPD that contained 24.8 and 7.5% milk casein-based protein,[6] for 5 days before use in the experiment, and were given in each diet and tap water *ad libitum* throughout the experiment. The composition of the diets was reported previously.[6] All experimental procedures were approved by the Ethics Committee on Animal Experiment of the National Institute for Minamata Disease (NIMD).

Determination of the Fate of MeHg —— MeHg was dissolved in saline and administered orally to rats at a dose of 20 μmol/kg on day 0. The animals were housed in metabolism cages (1 rat/cage), and urine and feces were collected for the following 24 hr after the administration. Other groups were housed in plastic cages (2–3 rats/cage) for 6 days. On day 1 or 6, the rats were sacrificed under pentobarbital anesthesia. Blood was collected from the inferior caval vein in heparinized syringes. After perfusion with ice-cold saline via the heart, the kidney, liver, testes and brain were excised. An aliquot of blood was centrifuged at 3000 rpm for 3 min to separate plasma. Hg content in each sample was determined by the oxygen combustion-gold amalgamation method[23] using a Rigaku Mercury Analyzer SP-3 (Nippon Instrument Co., Tokyo, Japan) and expressed as total Hg.

Another group of rats was used to examine the Hg distribution in plasma. Two hr after oral administration of MeHg (20 μmol/kg), blood was collected from the heart of each rat under ether anesthesia. An aliquot of blood was then centrifuged, and the obtained fresh plasma was immediately subjected to ultrafiltration using a Kurabo Centricut W-50 membrane filter at $5000 \times g$ for 4 min. Hg content in each filtrate [LMW fraction (less than 50000)] was determined as described above. Hg contents in the whole plasma, blood, kidney, liver and brain were also determined.

Preparation of Renal Brush Border Vehicles —— Renal brush border vehicles were prepared by the calcium precipitation method[24] with some modifications. Rats (6 for each dietary group) were sacrificed under ether anesthesia, and kidneys were excised. The fresh kidneys were minced and homogenized in ice-cold 0.1 M D-mannitol/0.1 M 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)-tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.4). 1 M $CaCl_2$ was added to each homogenate to obtain a final concentration of 10 mM. The homogenates were then centrifuged at $5000 \times g$ for 15 min in a Hitachi refrigerated centrifuge. The supernatants were carefully decanted and centrifuged again at $15000 \times g$ for 20 min. The pelleted materials were homogenized in ice-cold 0.1 M KCl/0.1 M HEPES-Tris buffer (pH 7.4) and centrifuged at $15000 \times g$ for 25 min. The pellets were resuspended in ice-cold 0.1 M D-mannitol/0.1 M HEPES-Tris buffer (pH 7.4) using a 1 ml syringe and a 26 G needle. Samples for enzyme assay were removed from the crude homogenates before the addition of $CaCl_2$ and from the resuspended brush border vehicles.

Enzyme Assay —— γ-GTP activity, an index of the brush border membrane, was determined by the

method of Orlowski and Meister[25] using L-γ-glutamyl-*p*-nitroanilide as a substrate. Protein content was determined by the method of Lowry *et al*.[26] using bovine serum albumin as a standard.

Transport Assays —— Uptake of ^{14}C-D-glucose and ^{14}C-L-phenylalanine into the brush border vehicles was measured using the Millipore filtration technique[27,28] with modification. Reaction mixture contained, in a total volume of 40 μl, 0.1 M D-mannitol, 10 mM HEPES-Tris buffer (pH 7.4), 1.0 or 0.2 mM substrate and 0.1 M NaCl or KCl. The brush border vehicles (20 μl) were pre-incubated at 25°C for 10 min. The reaction was started by adding 20 μl of substrate solution to the vehicles. After 20, 40, 60, 120, 300, and 1800 sec, the reaction was stopped by adding stop solution (1 ml), which contained 0.1 M NaCl, 0.1 M D-mannitol, 10 mM HEPES-Tris buffer (pH 7.4). For D-glucose uptake, 0.2 mM floridin-containing stop solution was used. Each sample diluted by stop solution was immediately filtered through a Millipore filter (HA, 0.45 μm), and the filter was then washed with 5 ml of ice-cold stop solution. After being dissolved in a liquid scintillator (FILTER-COUNT™, Packard Instrument Company, Inc., Meriden, CT, U.S.A.), radioactivity in each sample was measured by an Aloka Liquid Scintillation System LSC-3500.

Statistical Analysis —— Significant differences between individual means were determined by Student's *t*-test. Differences were considered significant at $p < 0.05$.

RESULTS

Influence of Dietary Protein Levels on MeHg Fate in Rats

After feeding either NPD or LPD for 5 days, body weight gain was 34.93 ± 3.13 and 0.43 ± 11.51 g in NPD- and LPD-fed rats, respectively. Despite the lower body weight in LPD-fed rats, no marked difference was observed in food intake (19.35 ± 2.37 and 17.52 ± 1.59 g/rat/day in NPD- and LPD-fed rats, respectively; mean ± S.D. obtained from 4 to 5 rats). We earlier reported no observable difference in the body weight of NPD- and LPD-fed mice.[6,7] Accordingly, the influence of the lowered dietary protein level on body weights would be different between rats and mice. Similar results in body weight changes in rats were reported previously.[29,30]

Figures 1 and 2 show tissue and excretory Hg after MeHg administration in NPD- and LPD-fed rats, respectively. The brain Hg concentrations were similar in the two dietary groups on both day 1 and 6 (Fig. 1). Hg concentrations in the kidney and testes were lower in LPD-fed rats than in NPD-fed rats on day 1, although the differences disappeared on day 6 (Fig. 1). Hg concentrations in the liver and blood were higher in LPD-fed rats than in NPD-fed rats, whereas plasma concentration was similar in the two dietary groups on both days (Fig. 1). Urinary Hg excretion was much lower in LPD-fed rats than in NPD-fed rats, whereas there was no significant difference in fecal Hg excretion between the dietary groups for 24 hr after MeHg administration (Fig. 2).

To account for the difference in the fate of MeHg between the dietary groups at 24 hr, Hg distribution in plasma was determined 2 hr after MeHg administration, as in mice.[7] Hg concentration in plasma LMW fraction was much higher in NPD-fed rats than in LPD-fed rats, whereas no concentration difference was observed in whole plasma (Fig. 3). The ratio of Hg concentration in the plasma LMW fraction to that in whole plasma was approximately twice higher in NPD-fed rats than in LPD-fed rats (Fig. 3). In contrast, Hg concentrations in the other tissues were similar in the two dietary groups (Table 1). Accordingly, the difference in Hg concentration in the plasma LMW fraction might give us hints regarding the reasons for the alterations in the fate of MeHg from 2 hr, at which there were no differences in Hg concentrations in several tissues, to 24 hr after MeHg administration, since MeHg metabolites in the plasma LMW fraction are a driving force in tissue uptake of MeHg.[13,22]

Influence of Dietary Protein Levels on Transport of a Typical Sugar and Amino Acid at Renal Brush Border Membrane

Renal brush border vehicles were prepared from the kidneys of 6 rats in each dietary group. Renal weight was lower in LPD-fed rats than in NPD-fed rats (1.87 ± 0.15 and 1.63 ± 0.08 g in NPD- and LPD-fed rats, respectively; mean ± S.D. obtained from 6 rats), as shown in mice.[6,7] γ-GTP activity, an index of the brush border membrane, was 1.59 and 1.68 IU/mg protein for the crude homogenates in NPD- and LPD-fed rats, respectively. The activity was approximately 13–15 fold higher in brush border membrane (23.07 and 22.12 IU/mg protein in the membrane from NPD- and LPD-fed rats, respectively) than in each crude homogenate. We earlier demonstrated that renal γ-GTP activity was identi-

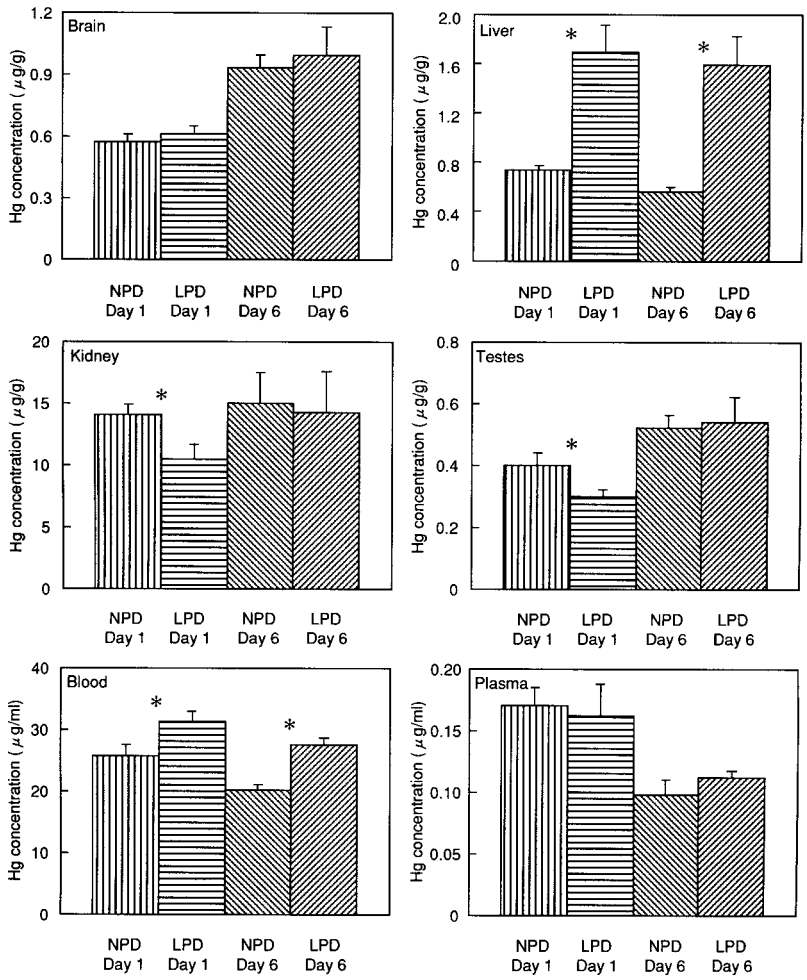

Fig. 1. Influence of Dietary Protein Levels on Tissue Hg Concentrations 1 and 6 days after MeHg Administration in Rats

Rats were orally administered MeHg (20 μmol/kg) on day 0. The values represent the mean ± S.D. obtained from 3 to 5 rats. Significantly different between the dietary groups on each specified day, $*p < 0.01$.

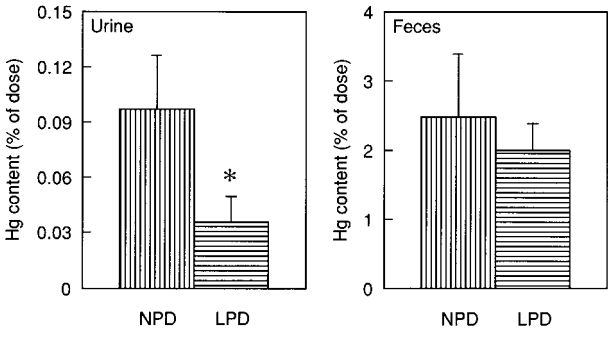

Fig. 2. Influence of Dietary Protein Levels on Excretory Hg for 24 hr after MeHg Administration in Rats

Rats were orally administered MeHg (20 μmol/kg) on day 0. The values represent the mean ± S.D. obtained from 4 rats. Significantly different from NPD-fed rats, $*p < 0.01$.

cal in NPD- and LPD-fed mice.[6] Therefore, this enzyme activity would not be susceptible to dietary protein deficiency.

Uptake of ^{14}C-D-glucose and ^{14}C-L-phenylalanine into the renal brush border vehicles obtained from NPD- or LPD-fed rats is shown in Fig. 4. In the presence of Na^+, uptake of both ^{14}C-D-glucose and ^{14}C-L-phenylalanine showed an overshoot phenomenon (Fig. 4A and 4B). After 30 min, uptake of ^{14}C-D-glucose was similar in each vehicle from NPD- or LPD-fed rats regardless of the presence of Na^+ (Fig. 4A). Similar results were obtained in the uptake of ^{14}C-L-phenylalanine (Fig. 4B). These results suggest that uptake of both the sugar and amino acid into the brush border vehicles reaches a plateau within at least 30 min, and the plateau levels at that time are not influenced by the levels of dietary protein. We then compared the initial uptake in the re-

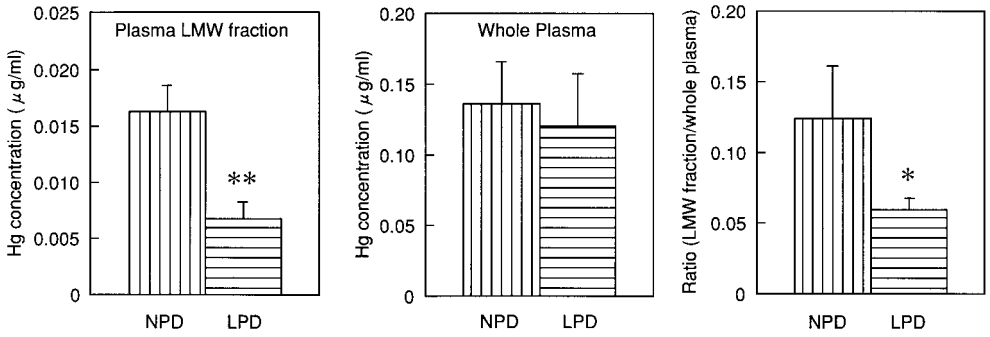

Fig. 3. Influence of Dietary Protein Levels on Distribution of Hg in Plasma 2 hr after MeHg Administration in Rats

Rats were orally administered MeHg (20 μmol/kg). The values represent the mean ± S.D. obtained from 3 to 5 rats. Significantly different from NPD-fed rats, $*p < 0.05$, $**p < 0.01$.

Table 1. Influence of Dietary Protein Levels on Tissue Hg Concentrations 2 hr After MeHg Administration in Rats

Tissue	Diet	
	NPD	LPD
Brain (μg/g)	0.16 ± 0.03	0.14 ± 0.04
Liver (μg/g)	1.38 ± 0.14	1.48 ± 0.21
Kidney (μg/g)	12.18 ± 1.24	10.33 ± 3.44
Blood (μg/ml)	11.92 ± 1.63	12.34 ± 2.01

Rats were orally administered MeHg (20 μmol/kg). The values represent the mean ± S.D. obtained from 3 to 5 rats.

nal brush border vehicles from both dietary groups. In the presence of Na^+, uptake of both ^{14}C-D-glucose and ^{14}C-L-phenylalanine for the first 20 sec was higher in the vehicles from LPD-fed rats than in those from NPD-fed rats (Fig. 4C and 4D). In the absence of Na^+, uptake of ^{14}C-L-phenylalanine was also higher in the vehicles from LPD-fed rats, whereas uptake of ^{14}C-D-glucose was identical in the vehicles in both dietary groups (Fig. 4C and 4D). Thus, the activity of neutral amino acid transport at the renal brush border membrane would be enhanced by dietary protein deficiency, as expected from the experiments using mice.[6] In addition, the transport activity of D-glucose would also be enhanced by the lowered dietary protein level, at least in the Na^+-dependent pathway.

DISCUSSION

The most important findings in this study using male rats are that urinary Hg excretion decreases (Fig. 2) and the uptake of ^{14}C-L-phenylalanine into the renal brush border vehicles is enhanced (Fig. 4D) by dietary protein deficiency. The decrease in urinary Hg excretion by the lowered dietary protein level is also observed in not only male mice[6,7,10] but also in female animals[31] despite the marked sex difference in the fate of MeHg in intact animals,[3,4] suggesting that this decrease would be universally caused by dietary protein deficiency. It has been suggested that urinary excretion of MeHg is regulated by many processes according to the following mechanism. A part of plasma LMW MeHg metabolites, mainly MeHg-L-cysteine and MeHg-GSH,[22] would influx into the proximal luminal space through glomerular filtration.[4] MeHg-GSH also influxes through the secretion system for GSH and its S-conjugates from the proximal tubule cells into the proximal luminal space.[4,20] MeHg-GSH influxed into the luminal space is rapidly degraded to MeHg-L-cysteine by γ-GTP and peptidases.[4,20,32] Although MeHg-L-cysteine is reabsorbed into the renal cells through the neutral amino acid transport system,[4] MeHg metabolites that escape reabsorption is excreted into urine.[4,20] Inhibiting γ-GTP by acivicin treatment markedly increases urinary Hg excretion with a concomitant decrease in renal Hg accumulation due to decreasing MeHg-L-cysteine, which would be a major form of MeHg metabolites reabsorbed.[4,20,32] Therefore, Hg excreted into the urine without reabsorption by acivicin treatment would reflect the influx of MeHg metabolites into the luminal space. The previous study using mice demonstrated that acivicin treatment markedly increased urinary Hg excretion and eliminated the difference, although urinary excretion in saline-treated controls was lower in LPD-fed mice than in NPD-fed mice.[6] In addition, there was no difference in renal γ-GTP activity in NPD- and LPD-fed mice.[6] Thus, influx and degradation of MeHg metabolites in the luminal space would be similar in NPD- and LPD-fed mice. These

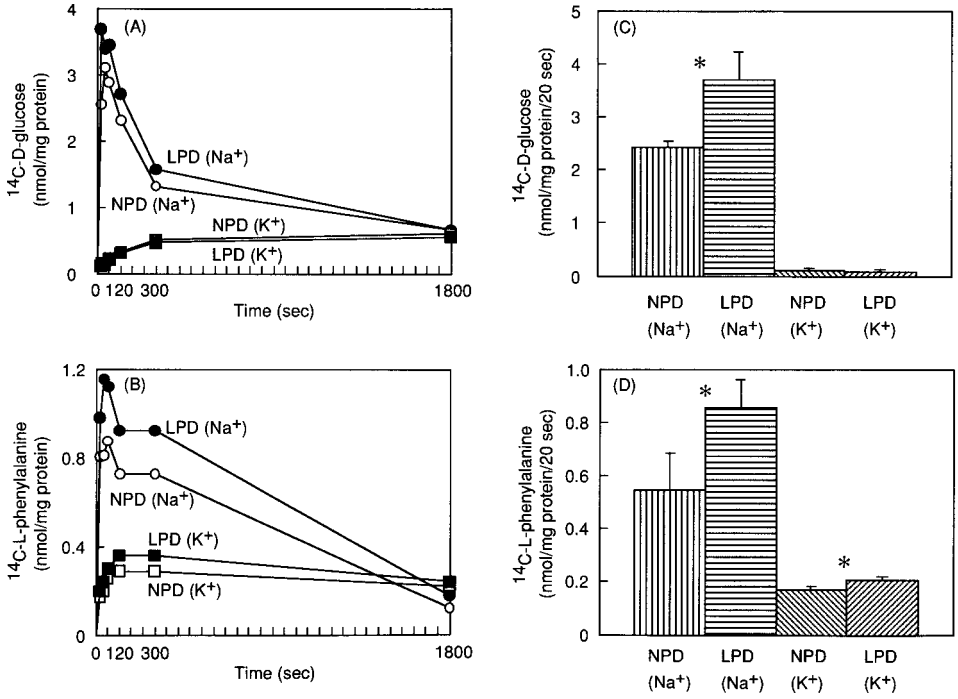

Fig. 4. Influence of Dietary Protein Levels on Transport of ^{14}C-D-Glucose and ^{14}C-L-Phenylalanine at Renal Brush Border Membrane in Rats

Uptake of ^{14}C-D-glucose (1.0 mM) and ^{14}C-L-phenylalanine (0.2 mM) into the renal brush border vehicles was measured using the Millipore filtration technique. (A, B) Representative data of time-dependent changes in uptake of ^{14}C-D-glucose (A) and ^{14}C-L-phenylalanine (B) into the renal brush border vehicles in the presence and absence of Na^+. (C, D) Initial uptake of ^{14}C-D-glucose (C) and ^{14}C-L-phenylalanine (D) into the renal brush border vehicles. The values represent the mean ± S.D. obtained from 3 to 5 determinations. Significantly different between the vehicles from rats fed each diet under the specified condition (in the presence or absence of Na^+), $*p < 0.05$.

results suggest that reabsorption of MeHg metabolites into the renal cells is a key step for the marked difference in urinary Hg excretion by dietary protein deficiency. However, there are few informations regarding dietary protein level-dependent alteration in reabsorption of LMW MeHg metabolites from the proximal luminal space. It is well known that L-phenylalanine competitionally inhibits the transport of MeHg metabolites such as MeHg-L-cysteine into the brain[12] and fetus.[33] In addition, the experiments using ^{14}C-L-phenylalanine revealed that the transport activity of neutral amino acids into the renal brush border vehicles was enhanced by the lowered dietary protein level (Fig. 4D). Accordingly, dietary protein deficiency would cause efficient reabsorption of MeHg metabolites such as MeHg-L-cysteine through the neutral amino acid transport system, and it would lead the decrease in urinary excretion of MeHg. On the other hand, Hg concentration in the plasma LMW fraction was lower in LPD-fed rats than in NPD-fed rats (Fig. 3), which might cause the lower glomerular filtration capacity of plasma LMW MeHg metabolites in LPD-fed rats. Therefore, this might also contribute the decrease in urinary Hg excretion in LPD-fed rats, although most MeHg metabolites would be reabsorbed after degradation as described above.

In spite of similarly decreasing urinary Hg excretion in both rats and mice, renal Hg concentration was suppressed by dietary protein deficiency in rats (Fig. 1) but similar in mice[7] at 24 hr. In addition, the percentage of Hg accumulated in the kidney in relation to Hg administered showed a similar tendency to the concentrations in both species (data not shown). These results appear to be contradictory to efficient reabsorption of MeHg metabolites into the renal cells from the proximal luminal space by dietary protein deficiency as described above. It has been suggested that hepatic GSH has an important role for renal transport of MeHg, since MeHg-GSH secreted from liver to circulation is transported to the kidney.[3,4,32] In addition, the efflux rate of GSH from the liver is closely related to renal accumulation (including urinary excretion) of MeHg.[3,4] Similarly, the efflux rate of GSH from the kidney is closely related to urinary excretion of MeHg.[3,4] Pre-

viously, we demonstrated that the efflux rate of GSH in the liver was lower in LPD-fed mice than in NPD-fed mice, and the rates in the kidney were identical.[6] Therefore, in mice, the decreased supplies from the liver to the kidney and efficient reabsorption of MeHg by dietary protein deficiency might lead to similar renal Hg accumulation. If dietary protein deficiency similarly influenced the GSH metabolism in rats as well as in mice, it might be possible to explain the difference in the renal Hg accumulation between the dietary groups of rats. On the other hand, Hg concentration in the liver as well as in the kidney in rats was markedly affected by dietary protein deficiency at 24 hr but not at 2 hr after MeHg administration (Fig. 1 and Table 1), although there were no significant differences in the concentrations in these tissues in mice at least within 24 hr.[7] The fact that, by dietary protein deficiency, Hg concentration in the plasma LMW fraction at 2 hr is suppressed in rats (Fig. 3) but is not affected in mice[7] gives rise to speculation us that plasma distribution of MeHg might be a key for the differences in Hg concentrations in these tissues at 24 hr. A support for this speculation is that LMW MeHg metabolites are a driving force in tissue uptake of MeHg.[13,22] Plasma distribution of MeHg is affected by the concentration of LMW thiol compounds and/or albumin,[22] to which most MeHg binds in plasma,[4,20] and also by the binding affinity of albumin to MeHg.[22] Thus, to understand the species difference in the influence of dietary protein deficiency on Hg accumulation in these tissues, further study on dietary protein level-dependent alteration in the metabolism of thiol compounds including GSH in rats would be necessary.

We previously reported a higher Hg concentration in the brain in LPD-fed mice than in NPD-fed mice.[6,7,10,31] In addition, the percentage of Hg accumulated in the brain to Hg administered was also higher in LPD-fed mice due to similar body and brain weights.[31] However, no difference was observed in the brain Hg concentration between the dietary groups in rats (Fig. 1). The percentage of Hg accumulated in the brain to Hg administered was much higher in LPD-fed rats than in NPD-fed rats, since body weight was lower in LPD-fed rats and the brain weight was similar in both dietary groups (data not shown). We have suggested that brain uptake of MeHg depends on the activity of the neutral amino acid transport system through which MeHg is transported into the brain, and the levels of circulating LMW MeHg metabolites which can readily enter the transport system.[7,22] The fact that NPD-fed rats showed a higher Hg concentration in the plasma LMW fraction than LPD-fed rats (Fig. 3) should lead to the lower brain Hg concentration in LPD-fed rats due to the similar brain weight, since MeHg metabolites in the plasma LMW fraction are a driving force in brain uptake of MeHg.[13,22] In contrast, as observed in mice,[7,10,11] it is expected that neutral amino acid transport into the brain would be more efficient in LPD-fed rats than in NPD-fed rats, which leads to the higher brain Hg concentration in LPD-fed rats. Thus, the two factors that affect uptake of MeHg into the brain might undergo the opposite modulations in the two dietary groups. Accordingly, a reason for the similar brain Hg concentration in NPD- and LPD-fed rats might be that the dietary protein level-dependent alterations in these two factors are balanced. On the other hand, Hg concentration in the plasma LMW fraction and in whole plasma were similar in NPD- and LPD-fed mice.[7] Accordingly, the more efficient transport of neutral amino acids into the brain by dietary protein deficiency[7,10,11] would lead to a higher brain Hg concentration in LPD-fed mice.[7,10]

In the present study, uptake of ^{14}C-L-phenylalanine and ^{14}C-D-glucose into the renal brush border vehicles would be enhanced by dietary protein deficiency (Fig. 4C and 4D). Dietary adaptations in intestinal absorption of nutrients, sugars and amino acids, have been studies in detail using brush border membrane *in vitro*,[21,34] and the following theoretical mechanism of the adaptations is found, although there are few reports using the renal membrane. Intestinal absorption for nutritionally non-essential, non-toxic solutes such as sugars increases at high dietary carbohydrate levels, since the caloric return justifies the expense of maintaining the transporter.[34] In contrast, absorption decreases at low dietary levels, since there is little return.[34] However, the theory of the adaptations for amino acid absorption is highly complex, since amino acids are used not only for calories as sugars but also as a source of nitrogen for proteins including transporters. Similar to sugars, absorption of amino acids increases at high dietary protein levels, although there is slight enhancement for absorption of essential amino acids which tend to be relatively toxic at higher concentrations compared with non-essential amino acids.[21] In nitrogen deficiency, maintaining absorption of essential amino acids is given priority, *i.e.*, absorption of essential amino acids increases or at least dose not decrease, but that of non-essential amino acids decreases.[21] Our present results using the renal brush

border vehicles appear to agree with this theory. Uptake of D-glucose into the renal vehicles was enhanced by feeding the LPD, which contained higher levels of carbohydrates such as cornstarch and sucrose than the NPD (Fig. 4C). In addition, the uptake of L-phenylalanine, an essential amino acid, was also enhanced by feeding the LPD that contained lower levels of protein than the NPD (Fig. 4D). Accordingly, dietary adaptations for the membrane transport of nutrients in the kidney might also be based on the theory demonstrated in the intestine.

The present results suggest that dietary protein deficiency enhances the neutral amino acid transport at the renal brush border membrane, which could lead efficient reabsorption of MeHg metabolites from the proximal luminal space, and this alteration might cause the marked decrease in urinary excretion of MeHg. It is also suggested that the concentration of circulating LMW MeHg metabolites as well as the neutral amino acid transport is affected by dietary protein levels, and this might be the key to the difference in the tissue accumulation of MeHg.

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