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Bioavailability of Cadmium from In Vitro Digested Infant Food Studied in Caco-2 Cells

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The solubility and bioavailability of cadmium (Cd) in infant foods, three cereal- and milk-based diets and two ready-to-use baby dishes, were studied after in vitro digestion and by using human intestinal Caco-2 cells. The solubility of Cd after in vitro digestion varied between diets; liver casserole had the highest solubility and was lower after infant as compared to adult digestion conditions. Generally, more Cd was soluble in infant intestinal than gastric juice in contrast to the results from the adult digestion. Caco-2 cells were incubated with supernatants of infant digests that had been equilibrated with ^{109}Cd during the in vitro digestion procedure, and cellular uptake and transport of ^{109}Cd were measured after 180 min. Statistically significant differences in both uptake and transport of Cd were detected between some of the diets and a control solution containing only digestive enzymes and $^{109}CdCl_2$. Uptake of soluble Cd in the cells varied between diets from 4 to 6%, and the transport over the monolayers was 1−2% of the dose. We conclude that age specific digestion conditions as well as composition of diets affect both solubility and bioavailability of Cd.

KEYWORDS: Cadmium; infant food; in vitro digestion; Caco-2 cells; bioavailability

INTRODUCTION

Food is the major exposure source of the toxic metal cadmium (Cd) to the majority of people (*1*), and half of the dietary intake originates from cereal products (*2*). Wheat has the highest Cd concentrations among grains, and unrefined wheat has higher levels than endosperm wheat because Cd accumulates in the outer part of the grain. Infants are exposed from an early age to Cd from cereal-based follow-on formulas and porridges, and cereal-containing infant foods might be a significant source of dietary Cd to the infant. In a previous study, we found up to 21 times higher mean Cd levels in cereal-based follow-on formulas as compared to cows' milk formulas, and the mean Cd intake in infants given follow-on formula was about half (*3*) of the provisional tolerable weekly intake (PTWI) of Cd, which corresponds to 7 μg/kg body weight (*4*). The PTWI is based on nephrotoxic effects of Cd because absorbed Cd accumulates in the kidney cortex and tubular kidney damage occurs at a certain critical concentration. However, animal studies indicate that the developing central nervous system in offspring may be more susceptible to Cd toxicity than the kidneys in adults (*5*, *6*).

The bioavailability of Cd in food is influenced by the composition of the diet and on the age and nutritional status of the individual. Fractional absorption of Cd from whole wheat diets is lower than from endosperm wheat diets in experimental animals (*7*), and iron deficiency is known to enhance the uptake of Cd in the gut (*8*). The gastrointestinal absorption of Cd in adults is approximately 5% (*9*), but in infants, it may be as high as 37% (*10*). The gastric pH in infants is higher than in adults. The intestinal epithelium is not fully developed, and the activity of most digestive enzymes is much lower in the young (*11*). Most likely, this will affect the bioavailability of Cd from different foodstuffs. The absorption of Cd in isolated intestinal segments of rats has been described as a two step process, involving a rapid internalization and a slow basolateral transport (*12*). A similar transport has been demonstrated in a human intestinal cell line model, Caco-2 cells (*13*, *14*).

The aim of the present study was to investigate the bioavailability of Cd from infant foods digested in vitro under infant gastrointestinal conditions. The soluble Cd in supernatants of the digested food samples was considered potentially available for absorption, and to verify this, the uptake and transport of the soluble Cd were measured in Caco-2 cells. The solubility of native Cd after in vitro digestion was also compared between infant and adult gastrointestinal conditions.

MATERIALS AND METHODS

Infant Food Samples. Five infant diets, recommended from 6 to 10 months of age, were bought at local retailers. Two were powdered infant porridges, one was a powdered follow-on formula, and two were ready-to-use baby foods in glass jars (**Table 1**). The fruit porridge contained 13% pear juice and 1% each of apple and apricot powder. The ready-to-use diets were thoroughly homogenized in a food processor until completely smooth before the in vitro digestion procedure.

Preparation of Gastric and Intestinal Juice. Porcine pepsin (catalog no. P-7000), porcine pancreatin (catalog no. P-1750), and bile

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Table 1. Infant Food Used in the In Vitro Digestion and Caco-2 Cell Experiments

infant food sample	main ingredients with decreasing weight contribution	native cadmium in food item (μg/kg)[a]
wholemeal porridge	whole wheat meal, skimmed milk powder, wheat flour, oatmeal	12.8
fruit porridge	oatmeal, skimmed milk powder, whole wheat meal, wheat flour, wheat bran (pear juice 13%, apple powder 1%, apricot powder 1%)	10.3
follow-on formula	skimmed milk powder, oatmeal, wheat flour	11.4
liver casserole	potatoes, green peas, beef liver, carrots	11.9
pasta Bolognese	beef, pasta of durum wheat, sweet pepper, green peas, wheat flour, onions	9.52

[a] Mean Cd levels of duplicate analyses from $n = 2$ for the ready-to-use diets and $n = 1$ for the powdered diets.

salts (catalog no. B-8756) were purchased from Sigma-Aldrich (Steinheim, Germany). NaCl pro analysi (p.a.), $NaHCO_3$ (p.a.), NaOH (p.a), HCl supra pur (s.p.), and HNO_3, 65% (p.a.) from Merck (Darmstadt, Germany) were also used in the in vitro digestion experiments.

Gastric Juice, Infant Conditions. Pepsin (0.5%, w/v) was in a 0.9% saline solution; the pH was adjusted to 5.5 with 1.5 M NaOH.

Gastric Juice, Adult Conditions. Pepsin (1%, w/v) was in a 0.9% saline solution; the pH was adjusted to 1.8 with HCl, 30%.

Intestinal Juice, Infant Conditions. Equal volumes of 1.5% (w/v) pancreatin and 0.075% (w/v) bile salts were in 0.9% saline solution.

Intestinal Juice, Adult Conditions. Equal volumes of 3% (w/v) pancreatin and 0.15% (w/v) bile salts were in 0.9% saline solution.

In Vitro Digestion Procedure. All glassware and utensils were soaked in 1.5 M HNO_3 overnight and rinsed six times in deionized, distilled water. The digestion procedure is a modification of a previous method by Crews et al. (*15*). Six samples per diet and gastrointestinal conditions were digested (24 samples/diet). Approximately 2 g of infant diet was weighed in polypropylene tubes. Gastric juice (10 mL/sample) was added, and the samples were incubated at 37 ° C for 4 h during gentle shaking. After 2 h, the pH was checked and adjusted to stay between 1.8 and 3.5 in the adult gastric juice and between 5.5 and 6.0 in the infant gastric juice by adding 4.75 M HCl and 1.0 M NaOH, respectively. After 4 h of gastric digestion, six samples per diet and infant and adult condition, respectively, were taken for a 60 min centrifugation at 2000*g* at 5 °C. The supernatants containing the soluble fractions of Cd were transferred to polypropylene tubes, weighed, and stored at 4 °C before Cd analyses. In the remaining samples, the pH was adjusted to 7.4 by addition of saturated $NaHCO_3$ solution. Intestinal juice (10 mL/sample) was then added, and the samples were again incubated at 37 °C for another 4 h digestion. After intestinal digestion, the samples were centrifuged as above. Blanks without infant food, only containing gastric and intestinal juices, were run in parallel.

Cd Analyses. A closed-vessel microwave combustion system, Milestone, MLS-1200-Mega (Sorisole, Italy), equipped with Teflon vessels and a temperature probe, was used for combustion of supernatants of the in vitro digests. Microwave-combusted supernatants were analyzed for Cd by atomic absorption spectrometry with graphite furnace technique and Zeeman background correction (Perkin-Elmer, 4100 ZL, Stockholm, Sweden).

The solubility of Cd, determined as Cd in the 2000*g* supernatants, was measured both after the gastric stage and after the intestinal stage. The supernatants (5 mL of gastric or 10 mL of intestinal) were weighed in Teflon vessels and microwave-combusted in 3 mL of HNO_3, 65%. One vessel was monitored by temperature control during a five step combustion program (**Table 2**). Furthermore, duplicate samples of the infant diets and the digestive enzymes were analyzed for native Cd in the same manner. An in vitro digestion control experiment was performed to examine whether Cd from the digestive enzymes stayed in solution or was bound to the food sample pellets after the in vitro digestion and centrifugation procedures. Briefly, native Cd was removed from four samples of the wholemeal porridge (approximately 2 g) by a two step washing procedure, first with 10 mL of 0.02 M ethylenediaminetetraacetic acid for 2 h and then with 10 mL of 0.03 M HCl solution for 1 h. After it was centrifuged, two pellets of the washed diets were analyzed for Cd, as described above, and two pellets were incubated with digestional juices for 2 h before centrifugation and Cd analysis of the pellets.

Table 2. Five Step Microwave Combustion Program for Supernatants of In Vitro Digested Infant Foods

step	time (min)	power (W)	temp (°C)
1	5	250	121
2	3	400	140
3	3	600	160
4	4	850	180
5	3	650	175

Quality Control of the Cd Analyses. Two certified reference materials were analyzed for Cd in parallel with the infant diets: wheat flour (GBW 08503 Cereal and Oil Chemistry Research Institute, Ministry of Commerce, Beijing, China) and lyophilized bovine liver (BCR 185, no. 1060, Community Bureau of Reference—BCR, Brussels, Belgium). The result (means ± SD) for the wheat flour was 30.8 ± 1.1 ($n = 4$) and for the bovine liver was 293 ± 97 μg/kg dry weight ($n = 2$), which agreed well with the respective certified values of 31 ± 4 and 298 ± 25 (mean ± 95% CI). Our laboratory also participated with satisfactory results (mean *Z* score, −0.7, $n = 4$) in a proficiency testing of trace element in foods, arranged by the Swedish National Food Administration.

Caco-2 Cell Experiments. Before the bioavailability experiments in the Caco-2 cells, the diets were digested in gastric and intestinal juices under infant conditions as described above. Radioactive $^{109}CdCl_2$ with a specific activity of 0.7 mCi/μg, from Amersham Pharmacia Biotech (Uppsala, Sweden), was added to each sample of 1 g of diet or 0.06 g of enzymes, immediately after the addition of 5 mL of gastric juice, allowing native and radioactive Cd to equilibrate during the 2 × 4 h digestion period. The added radioactivity ranged from 0.4 to 0.7 μCi/sample. After complete digestion, the samples were centrifuged as described above and ^{109}Cd was determined in the supernatants.

Caco-2 cells were generously provided by Dr. Per Artursson at the Department of Pharmacy, Uppsala University. The cells were grown in Dulbecco's Modified Eagle medium (DMEM) (Life Technologies, Täby, Sweden), containing 10% v/v fetal calf serum (Biotech Line AS, Gothenburg, Sweden), 10 mM HEPES, and 50 μg/mL Gentamicin (Life Technologies). The cells were maintained at 37 °C in an incubator with 5% CO_2/95% air atmosphere at 95% relative humidity. Cells between passages 102 and 104 were harvested at 80% confluence and seeded onto semipermeable filters (Transwells, ∅ 12 mm, pore size 0.4 μm) at a density of 500 000 cells/cm^2 and allowed to differentiate on the filters for 21 days before the bioavailability experiments. The medium was changed every second day.

Control Experiment—Integrity of the Monolayers. To investigate possible adverse effects of food components or digestive enzymes in the supernatants on the Caco-2 cells, the integrity of the cells was carefully assessed in a control experiment prior to the bioavailability experiments. Caco-2 monolayers were incubated with supernatants that had been heat-treated for 4 min at 100 °C for inactivation of the digestive enzymes (*16*) or with corresponding supernatants that had not been heat-treated ($n = 4$/diet/treatment).

The transepithelial electrical resistance (TEER) was measured across the monolayers every 45 min for 270 min with a Millicell ERS device (Millicell ERS, Millipore, Bedford, MA). Background resistance was determined by measuring the TEER across a filter without cells, with enzyme blank supernatant on the apical and DMEM on the basolateral side. At the same time points, 10 μL aliquots were taken from the apical side of the monolayers to determine release of lactate dehydrogenase (LDH). LDH was quantified spectrometrically by using a kinetic determination procedure according to the instructions of the manufacturer, Sigma Diagnostics, 340-LO (Stockholm, Sweden). The TEER and LDH values were compared between monolayers incubated with heat-treated and nontreated supernatants. To avoid bacterial contamination, the TEER measurements and the sampling for the LDH analyses were performed in an LAF bench with the filter plates kept on a heated plate calibrated to maintain the temperature of the medium at 37 °C.

Bioavailability Experiments—Cellular Uptake and Transport of Cd. On the basis of the results of the integrity control experiment, the bioavailability experiments were carried out for 180 min at 37 °C with 1.5 mL of DMEM on the basolateral side and 0.5 mL of nontreated supernatant of the infant food digests containing ^{109}Cd on the apical side. Ten microliter aliquots were taken from the apical side for LDH analysis immediately after applying the supernatants, as described in the integrity control experiment, and again after 180 min of incubation. At these time points, TEER across the monolayers was also checked. Monolayers with resistances <150 Ω/cm^2 were not used in the bioavailability experiments. At the end of the experiment, the filter supports with the monolayers were taken from the basolateral chamber and rinsed twice with 2 mL of 4 °C DMEM medium before lyzed in 5 mL of 0.5 M NaOH overnight. The basolateral solution was collected, and the radioactivity in the cells and in the basolateral solution was counted in a γ-counter. Eight monolayers per diet were analyzed.

Statistics. Data are presented as medians and interquartile ranges (IQR). The Kruskall-Wallis and Scheffé's post-hoc tests were used in the bioavailability Caco-2 cell experiments and to make comparisons in Cd solubility between diets digested under the same gastrointestinal conditions. In the integrity control experiment, the nonparametric Wilcoxon signed rank test was used to analyze changes in TEER and LDH between time 0 and 270 min in monolayers incubated with nontreated and heat-treated supernatants, respectively. The Mann−Whitney U test analyzed differences in LDH release from monolayers incubated with nontreated and heat-treated supernatants at times 0 and 270 min. The Mann−Whitney U test also analyzed differences in Cd solubility between infant and adult digestion of a diet and between gastric and intestinal digestion of a diet. The level of significance was set to $p < 0.05$.

RESULTS

Native Cd levels were low and similar in the infant diets (**Table 1**), and the distribution of ^{109}Cd was similar to that of the native Cd after the in vitro digestion and centrifugation procedures (data not shown). The pepsin and pancreatic enzymes contained 10.6 and 32.7 μg Cd/kg powder, respectively. Cd in the bile salts could not be detected. The contribution of Cd from the enzymes in the in vitro digestion experiments corresponded to 12−16% of the total Cd content in the diet samples. The results from the in vitro digestion control experiment showed that released Cd from the enzymes did not bind to the pellets but stayed in solution after digestion and centrifugation. The Cd concentration in the supernatants of the blanks, containing only the digestive enzymes, was therefore subtracted as background from the Cd concentration in the supernatants of the digested food samples.

The solubility of Cd was significantly lower after infant digestion, with the higher pH in the gastric juice and the lower concentration of digestive enzymes, than after adult digestion (**Figure 1A**,**B**). Only the pasta Bolognese had comparable amounts of Cd in the supernatants after complete digestion with approximately 30% solubility in both adult and infant intestinal juices. Infant digestion conditions rendered significantly less soluble Cd in the gastric juice than in the intestinal juice in the cereal-containing diets. Approximately 30% was soluble in the intestinal juice as compared to 1−19% in the gastric juice. The liver casserole made an exception in the infant digestion and had about the same solubility in gastric and intestinal juice, 63 and 52%, respectively. In contrast, under adult conditions, significantly more Cd was soluble in the gastric juice as compared to the intestinal juice for all diets. The Cd solubility also differed between the diets when they had been digested under the same gastrointestinal condition regarding enzyme concentration and pH of the digestion juices (**Figure 1A**,**B**). The liver casserole had significantly higher solubility than the other diets in the infant digestion. The wholemeal porridge and the follow-on formula had very low Cd solubility in infant gastric juice, 1 and 8%, respectively.

The results from the integrity control experiment showed that the TEER varied between the diets but was stable for 270 min (**Figure 2A**,**B**). There were no statistically significant differences in TEER between time 0 and 270 min in the monolayers, regardless of treatment of the supernatants ($p = 0.13$ in nontreated and $p = 0.27$ in heat-treated supernatants). However, the LDH release from monolayers incubated with heat-treated supernatants was significantly higher than the release from monolayers incubated with nontreated supernatants ($p = 0.02$ at time 0 and $p = 0.0006$ at time 270 min). In addition, the LDH release from monolayers incubated with heat-treated supernatants increased significantly ($p = 0.0013$) during the experimental period (270 min) whereas the LDH release from monolayers incubated with nontreated supernatants remained stable ($p = 0.11$) during the entire experimental period (**Figure 2C**,**D**). Similar TEER and LDH results were obtained in the bioavailability experiments (data not shown) as in the integrity control experiment.

The Cd uptake in the Caco-2 cells incubated with supernatants of the infant food or enzyme control varied between 3.8 and 6.3% of the dose (**Table 3**). Liver casserole and fruit porridge had significantly higher uptake of Cd as compared to wholemeal porridge and enzyme control. Also, the Cd transport over the monolayers was dependent on the diet, varying from 0.9 to 2.1% of the dose. Significantly higher transport was seen over monolayers incubated with supernatants of digested follow-on formula or pasta Bolognese as compared to control monolayers incubated with enzyme solution.

DISCUSSION

To improve the risk assessment for Cd in children, relevant models for studying the bioavailability of Cd in foods are essential. In the present study, we have combined in vitro digestion with exposure of the soluble Cd from the digests to human intestinal cells under strictly controlled conditions. As compared to in vitro digestion alone, which measures only the solubility, this gives a better estimate of Cd availability. The Caco-2 cell line used in the present study shows many of the functional and morphological properties of human enterocytes (*17*, *18*). The cells differentiate into polarized enterocyte-like monolayers, develop microvilli, and act similarly to small intestinal epithelial cells. To our knowledge, no previous investigations have simulated infant digestion conditions and studied the uptake and transport of realistically low levels of soluble Cd from infant food in fully differentiated human enterocytes.

In the present in vitro digestion experiments, infant digestion generally resulted in lower Cd solubility as compared to adult

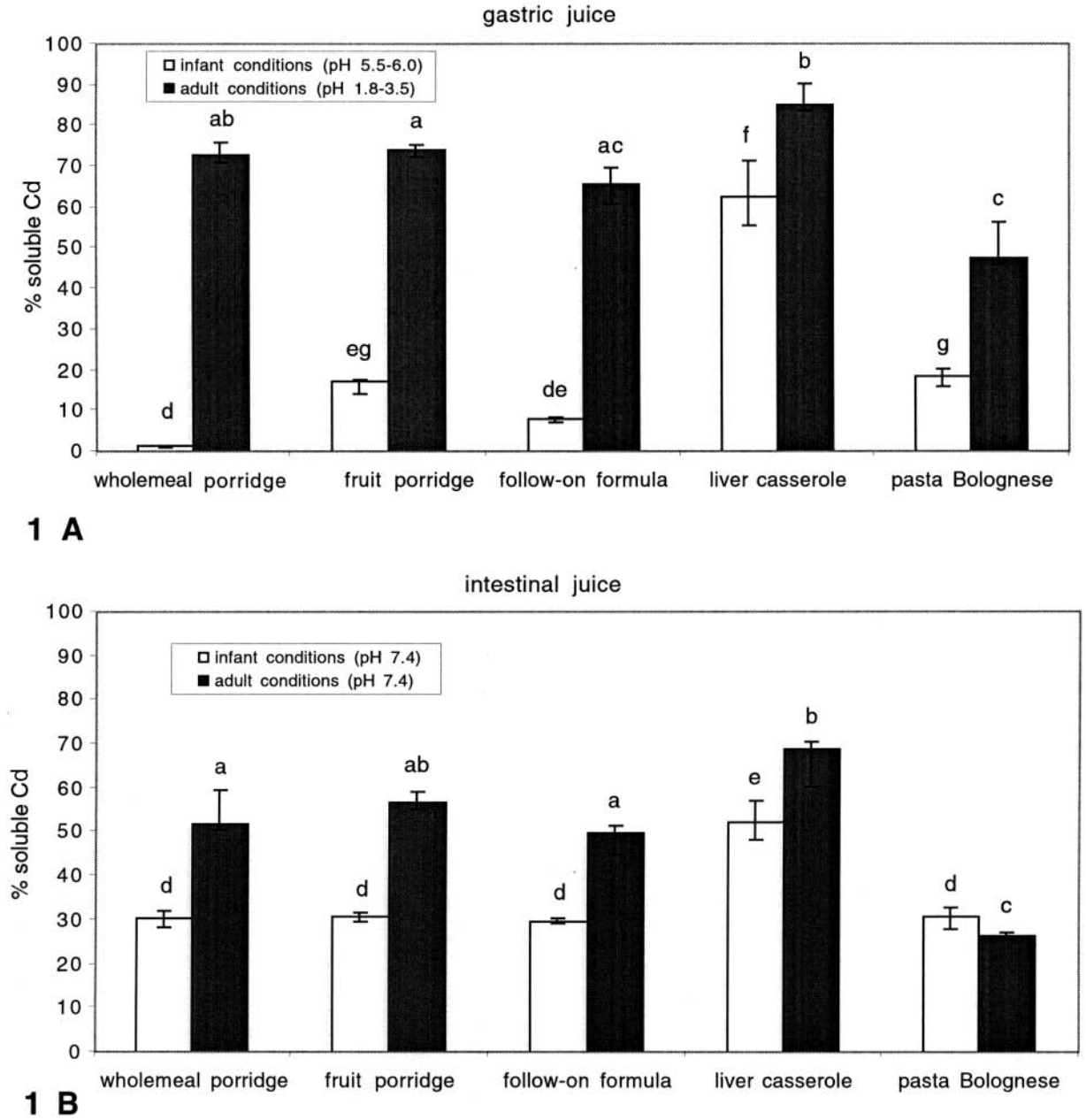

Figure 1. (**A**,**B**) Fractional solubility of cadmium after in vitro digestion of infant food. Gastrointestinal conditions were adjusted to simulate infants and adults. The pepsin concentration in infant and adult gastric juice was 0.5 and 1.0%, respectively. Infant intestinal juice contained 0.08% bile salts and 1.5% pancreatic enzymes, and adult intestinal juice contained 0.15% bile salts and 3% pancreatic enzymes. Results are expressed as medians and interquartile ranges $n = 6$. Bars not sharing a letter indicate significantly different Cd solubility between diets digested under the same gastrointestinal condition.

digestion of the diets. The higher pH in the infant gastric juice and the lower enzyme concentrations as compared to adult conditions probably explain this as it means less dissociation of Cd from food components and less proteolytic degradation with changes in binding sites for Cd. For example, pepsins have a pH optimum of 1.5−3.5 (*19*), which makes the pH of the infant gastric juice (5.5−6.0) too high for the pepsin to be fully active. The higher solubility in infant intestinal as compared to gastric juice could be explained by a higher degree of degradation of Cd binding food components in the intestinal juice as the pH for the pancreatic enzymes was optimal.

We also found differences in Cd solubility between the diets when they had been digested under the same gastrointestinal condition. Different Cd levels in the diets cannot explain this since the native Cd levels were similar. Different ingredients and binding sites for Cd are probably the reason for this. The liver casserole, for example, had the highest solubility of native Cd at infant digestion conditions, 63 and 52%, respectively, in the gastric and intestinal juice, whereas the solubility of Cd from the wholemeal porridge was only 1% in the infant gastric juice. The whole wheat meal in the wholemeal porridge contains phytic acid and insoluble dietary fiber such as lignin and cellulose. Phytic acid forms strong complexes (phytates) with polyvalent metal ions, in food as well as in the gut (*20*), and the ability to digest phytates is very limited in the human small intestine. The binding of Cd to dietary fiber is pH-dependent (*21*), and the minimum solubility for Cd−phytate complexes is found at pH 6 (*20*), which corresponds to the pH of our infant gastric juice. Cd bound to insoluble fibers or phytic acid would consequently be removed by the centrifugation after the gastric

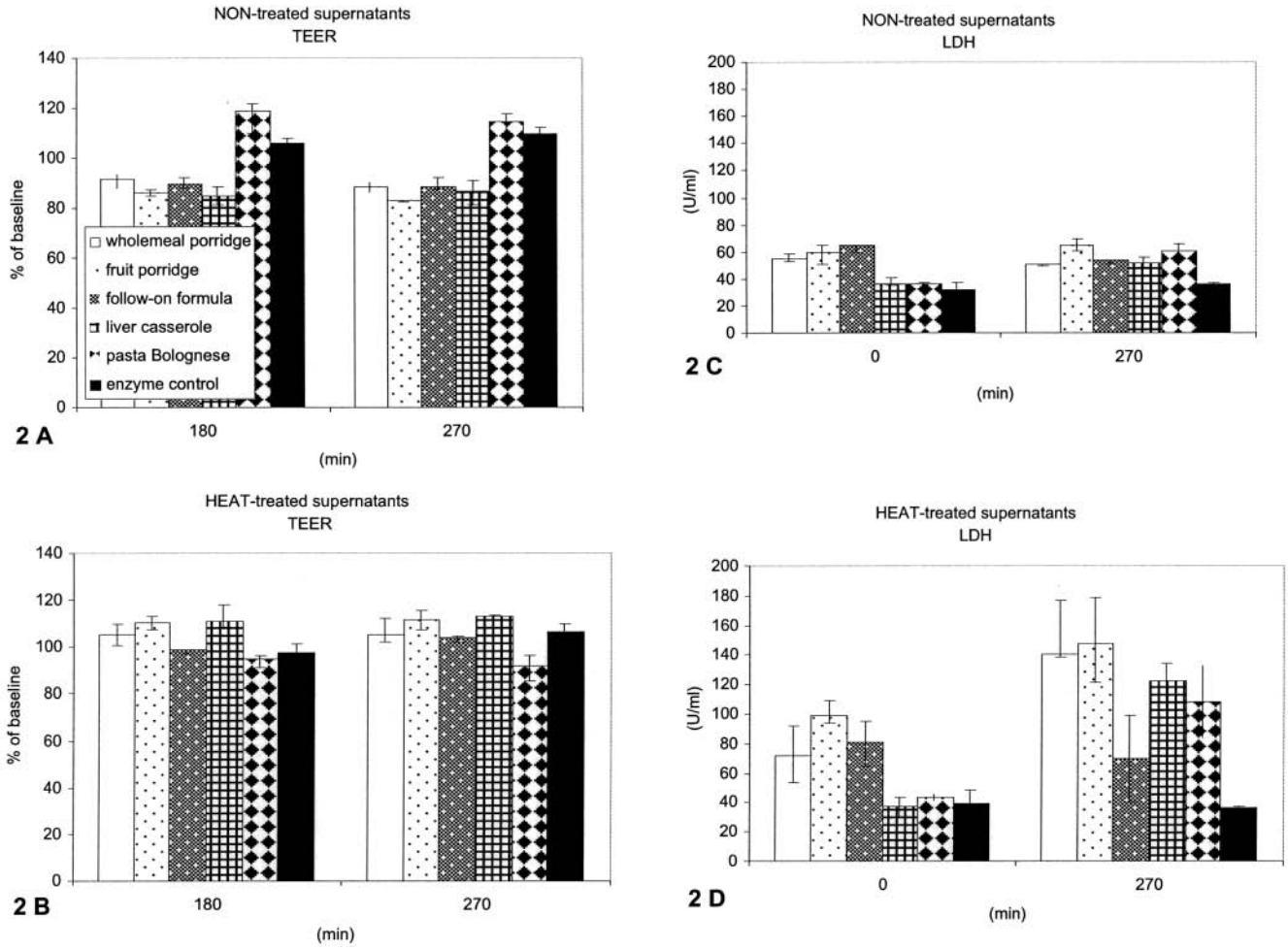

Figure 2. (**A**–**D**) Measurements of TEER across Caco-2 cell monolayers and LDH release in the apical medium after incubation at 37° C with nontreated or heat-treated (100 °C for 4 min) supernatants of in vitro digested infant food. TEER values are expressed as % of the values at time 0. Data are expressed as medians and IQRs of 3–4 monolayers/diet/treatment. No statistically significant effects were seen in TEER over time in any of the treatment groups. However, LDH release was significantly higher after incubation with heat-treated than with nontreated supernatants and increased also significantly over time after incubation with heat-treated supernatants.

Table 3. Uptake and Transport of Cd in Caco-2 Cells after 3 h of Incubation with Supernatants of In Vitro Digested Infant Foods[a]

	wholemeal porridge	fruit porridge	follow-on formula	liver casserole	pasta Bolognese	enzyme control
exposure/monolayer (pmol)[b]	11.8	12.1	9.90	8.45	9.65	3.40
uptake (pmol) (% of dose)	0.446, 0.09 (3.79) b	0.766, 0.08 (6.34) a	0.466, 0.16 (4.71) ab	0.495, 0.11 (5.86) a	0.542, 0.09 (5.62) ab	0.128, 0.03 (3.76) b
transport (pmol) (% of dose)	0.156, 0.05 (1.32) ab	0.237, 0.08 (1.96) ab[c]	0.209, 0.04 (2.11) b	0.141, 0.08 (1.67) ab	0.194, 0.19 (2.01) b	0.03, 0.02 (0.88) a

[a] Results are expressed as medians and IQRs (n = 6–8). Different letters indicate significant differences between diets as % of dose. [b] Native Cd from diet + Cd from enzymes + ^{109}Cd. [c] p = 0.05 as compared to enzyme control.

stage and explain the extremely low solubility of Cd in the wholemeal porridge in the infant gastric juice.

As demonstrated in the Caco-2 cell experiments in the present study, the compositions of the infant diets affected the bioavailability of Cd. Monolayers incubated with supernatants of liver casserole or fruit porridge had significantly higher intracellular uptake of soluble Cd as compared to wholemeal porridge and enzyme control solution. Soluble Cd in beef liver is mainly bound to metallothionein (MT), a low molecular weight protein with high affinity for Cd (*22*). The concentration of H^+ is the major factor known to influence the dissociation of metals from MT and almost all Cd bound to MT is released at pH 3 (*23*). However, metals begin to dissociate from MT already when the pH drops from 7 to 5. Hence, the pH of our infant gastric juice would be low enough for sufficient dissociation of Cd from MT. Furthermore, MT with no metals bound to it is extremely susceptible to degradation by proteolytic enzymes (*24*). Thus, in the present study, it is possible that Cd have dissociated from MT in sufficient amounts in the infant gastric juice and that the proteolytic enzymes have degraded most of the MT. Free Cd ions in the supernatant of the intestinal liver casserole digest could then bind electrostatically to the cell membranes of the Caco-2 cells and be internalized (*12*). In the cells, the free Cd ions may bind to intracellular components such as glutathione or other molecules containing SH groups and this may delay the transepithelial transfer of Cd to the

basolateral side. The low uptake of soluble Cd from the wholemeal porridge in the Caco-2 cell monolayers is in agreement with a previous in vivo study where we observed significantly lower intestinal retention of $^{109}CdCl_2$ given to rat pups as a single oral dose in a wholemeal formula as compared to $^{109}CdCl_2$ given in water (*25*). Lower fractional absorption of Cd has also been reported in rats given whole wheat diets as compared to rats given endosperm wheat diet (*7*) or sugar beat- or carrot-based diets (*26*). The role of phytic acid and its complex formation with Cd for the bioavailability of Cd needs to be further investigated.

It could be suspected that the digestive enzymes or food components in the supernatants of the digests would affect the Caco-2 cells, and this was carefully investigated in a control experiment with heat-treated supernatants prior to the bioavailability experiments. Because of the elevated LDH levels caused by the heat-treated supernatants, nontreated supernatants were used in the bioavailability experiments.

The results of the present Caco-2 cell experiments can be compared to a previous study with similar experimental conditions by Tallkvist et al. (*27*) where fully differentiated Caco-2 cells were incubated with 25 nM Cd as $^{109}CdCl_2$ for 180 min at 37 °C. Our results, from similar Cd concentrations, show lower intracellular uptake and higher transport of Cd as compared to the previous study. It can be speculated that intracellular uptake of Cd is inhibited by food components and that food-bound Cd interacts with intracellular components to a lesser extent than free Cd ions do. This may shorten the intracellular time period for food-bound Cd and facilitate the transport over the cells. This is in agreement with the observed higher intracellular uptake of the released Cd from MT in the liver casserole, as was discussed above.

The results of the present study show that the solubility of an element does not always correlate with its bioavailability. The fractional solubility of Cd after the infant digestion ranged from 1 to 63%, but the fractional uptake or transport was generally much lower, varying from 1 to 6%. Cd in the liver casserole had the highest solubility and the highest intracellular uptake, which may indicate a higher risk of exposure due to both a higher Cd dose accessible to the intestinal cells and a higher cellular uptake. However, the Cd solubility from the follow-on formula was very low, only 8% in the infant gastric juice. Still, the transepithelial transport of the soluble Cd from the formula was significantly higher as compared to the enzyme control solution.

In vitro digestion in combination with exposure of Caco-2 cell monolayers to supernatants of the food digests seems to be a promising model for studying the bioavailability of realistically low Cd levels in food. The uptake and transport of Cd in the Caco-2 cells in the present study ranged from 1 to 6% and is in agreement with the approximate 5% gastrointestinal absorption of Cd from food (*9*). The differences in bioavailability of Cd from the infant diets were small but significant in the Caco-2 cells. Incubation periods longer than 180 min might have enabled detection of larger differences.

However, interpretations of in vitro results must be done with caution. Cd bound to food components is indeed less water soluble than $CdCl_2$, but enzymes and acids of the digestive tract may degrade these complexes making Cd available for absorption. This has been considered in the present model. However, other aspects need to be considered, for instance, effects of the bacterial flora in the human colon that potentially degrade a significant proportion of the dietary fibers. Cd bound to the fiber in vitro might thus be released during bacterial degradation and be absorbed in vivo. Furthermore, the gastrointestinal absorption of Cd in infants might be higher than our results indicate, as the intestinal epithelium of infants is not fully developed with looser tight junctions as compared to adults, which could not be simulated in the Caco-2 cell model. Also, the enterocyte turnover is lower in infants than in adults, which might lead to longer retention periods of Cd in the gut mucosa with increased risk for Cd to be absorbed.

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