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Chronic oral administration of rhamnogalacturonan-II dimer, a pectic polysaccharide, failed to accelerate body lead detoxification after chronic lead exposure in rats

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Lead is a ubiquitous heavy metal and its toxicity remains an important public health issue. In previous work, we reported that ingestion of rhamnogalacturonan-II dimer (dRGII), a pectic polysaccharide, may decrease intestinal absorption and status of Pb in rats. Here, we evaluated the potential detoxifying effect of different doses of dRGII after chronic oral Pb exposure in rats. For this purpose, six groups of ten male Wistar rats weighing 150 g were treated as follows: group A received a semi-purified control diet for 6 weeks; groups B, C, D, E and F received the same diet plus 3 mg Pb (as acetate) for 3 weeks. Group B was then killed. Groups C, D, E, and F continued to receive the semi-purified control diet containing 0, 2, 6 or 18 g dRGII/kg diet for 3 additional weeks. During the last 5 d, a Pb conventional balance study was performed. Rats were then anaesthetized and tissues were sampled for Pb and essential minerals assay. The results showed that residual Pb in the added dRGII was not available for absorption. However, the added dRGII failed to induce any significant increase in faecal or urinary Pb excretion. Consequently, at the end of the study the intestinal Pb absorption and balance remained unchanged in the animals receiving the different doses of dRGII. In line with this, we showed that dRGII administration was not effective in decreasing tibia or kidney Pb levels in rats. In conclusion, Pb complexed by dRGII in fruits and vegetables and fruit juice is thus mostly unavailable for intestinal absorption. However, the addition of dRGII after chronic Pb exposure does not help Pb detoxification.

Rhamnogalacturonan-II: Lead absorption: Lead detoxification: Rat

Lead is a ubiquitous and heavy metal, and its toxicity remains an important public health issue (Silbergeld, 1996; Markowitz, 2000). Some of the chelating agents used in Pb detoxification therapy are known to have adverse effects and to promote losses of essential cations (Thomas & Chisolm, 1986; Chisolm, 2000). The search for more specific and safer agents for the prevention and treatment of Pb poisoning is thus still justified.

Since the 1970s, the metal-binding capacities of some pectin cell-wall polysaccharides have been investigated in an attempt to use their metal-binding properties in the detoxification of heavy metals such as Pb and Cd (Niculescu *et al.* 1968; Rose & Quarterman, 1987). Most of the reported work on the action of pectin on Pb poisoning has shown that Pb absorption and status in rats are lowered by adding pectin to the experimental diets (Bondarev *et al.* 1979; Kushneva & Koltunova, 1997).

Recently, rhamnogalacturonan-II dimer (dRGII) has been isolated and characterized from apples and wine (Pellerin *et al.* 1996). dRGII is a complex pectic polysaccharide present in fruits and vegetables and also in fruit juices and wine. *In vitro* studies have shown that dRGII forms coordination complexes with di- and trivalent cations. The chelation of cations by dRGII is very specific; it binds Pb^{2+}, Sr^{2+}, and Ba^{2+} but not essential cations such as Mg^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}. In our previous work (Tahiri *et al.* 2000), we showed that Pb was less bioavailable from the complex Pb–dRGII than from Pb acetate. We have also shown that the addition of unleaded dRGII to their diet helped further decrease Pb absorption and burden in rats. These data

Abbreviation: dRGII, rhamnogalacturonan-II dimer.

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strongly suggest a potential detoxifying effect of dRGII, probably through chelation of dietary and endogenous Pb in the gastrointestinal tract. The aim of the present study was therefore to evaluate the potential detoxifying effect of different doses of dRGII after chronic Pb exposure in rats.

Materials and methods

Reagents and equipment

Suprapure HNO_3 and H_2O_2 were purchased from Merck (Darmstadt, Germany) and Pb acetate was purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals were of the highest quality available. Distilled water was used throughout. An inductive coupled plasma mass spectrometer (Plasmaquad II system; Fisons Instruments; Manchester) with a Meinhard nebulizer was used for the Pb assay and a Perkin Elmer AA800 spectrometer (Perkin Elmer, St-Quentin en Yvelines, France) for the Ca, Mg, Fe, Zn and Cu assays.

Rhamnogalacturonan-II dimer preparation

dRGII was prepared from an industrial apple residue (Les Vergers de Chateaubourg, 35220 Chateaubourg, France). Apple residue was treated for 3 d with pectinolytic enzymes (Rapidase Liq$^+$, 0·08 % (v/v); Gist-Brocades, Seclin, France). The supernatant fraction was filtered and concentrated, and then injected on a DEAE-Fractogel 650M column (18 × 25 cm; Pharmacia) equilibrated at 150 ml/min with 30 mmol acetate buffer/l, pH 5. The dRGII purified fraction was obtained by washing the column with 200 mmol NaCl/l in 30 mmol buffer acetate/l, pH 5. The purified dRGII was then injected on a Superdex 75HR column and the glycosyl residue composition was determined (Pellerin *et al.* 1996) to verify the homogeneity of the fraction. The structure of dRGII is shown in Fig. 1.

Animals and diets

Male Wistar rats weighing 150 g from the laboratory animal facility of the National Institute of Agronomic Research (INRA de Clermont-Ferrand Theix) were used. They were housed under conditions of constant temperature (20–22°C), and humidity (45–50 %) in rooms with a fixed 12-h artificial light–dark cycle. The rats were cared for according to the guidelines of the European Community for the use of experimental animals (L358-86/609/EEC). All the rats were first adapted using a semi-purified diet for 7 d. The diet used was that recommended by the Ad Hoc Committee on Standards for Nutritional Studies in Animals (Reeves *et al.* 1993). Its composition is given in Table 1. Measured dietary levels of Ca, Mg, Fe, Zn and Cu in this diet were 5540, 540, 39·8, 43·1 and 6·6 mg/kg dry weight, respectively. After the adaptation period, the sixty rats were randomized into six groups. These groups comprised a control group (group A) receiving the control diet, and five groups receiving 3 mg Pb/kg diet as Pb acetate for 3 weeks. After these 3 weeks, Pb addition was stopped. One group of ten Pb-treated animals (group B) was killed to serve as a baseline for Pb status. The control group continued to receive the semi-purified control diet for 3 additional weeks. The four leaded groups (groups C, D, E and F) received rations supplemented with one of the following doses of dRGII: 0, 2, 6 or 18 g/kg diet respectively for 3 additional weeks. During the last 5 d of the experimental period the rats were placed in individual

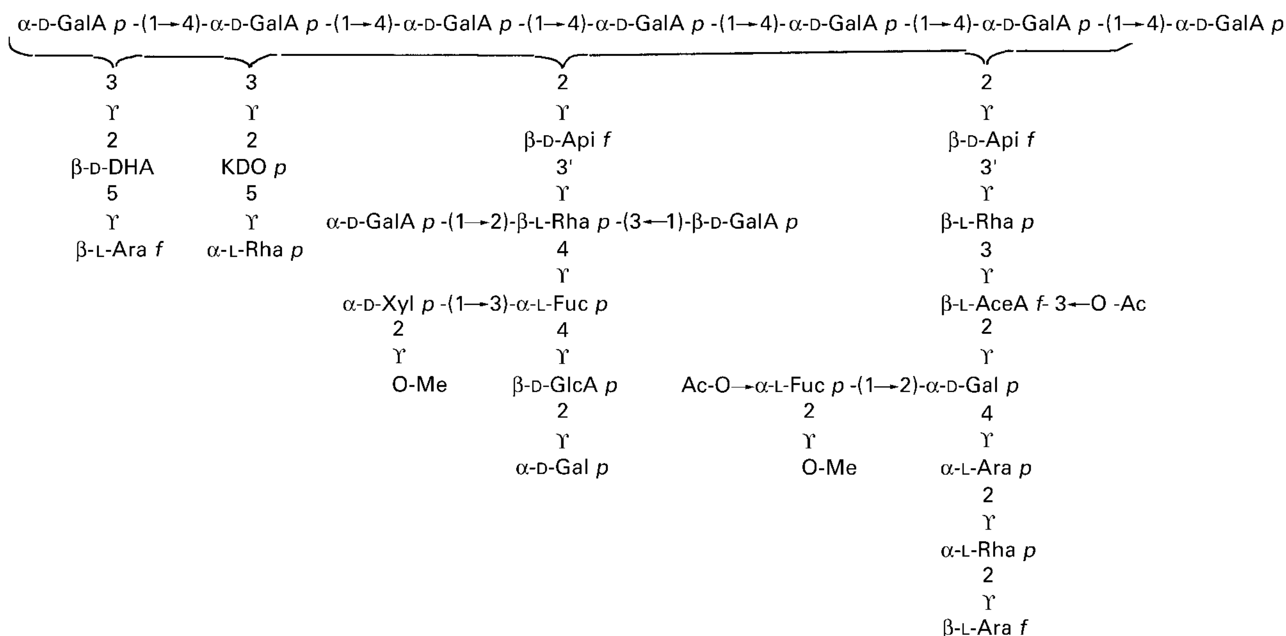

Fig. 1. Structure of the rhamnogalacturonan-II monomer. The four oligoglycoside side chains A–D are shown whose residues are numbered according to the model sequence. 2-O-Me-α-L-Fuc *p*, 2-O-methyl-α-L-fucopyranose; α-L-Rha *p*, α-L-rhamnopyranose; α-L-Fuc *p*, α-L-fucopyranose; 2-O-Me-α-D-Xyl *p*, 2-O-methyl-α-D-xylopyranose; α-L-Ara *p*, α-L-arabinopyranose; β-L-Ara *f*, β-L-arabinofuranose; β-D-Api *f*, β-D-apiofuranose; α-D-Gal *p*, α-D-galactopyranose; α-D-GalA *p*, α-D-galacturonic acid; β-D-GlcA *p*, β-D-glucuronic acid; KDO, 3-deoxy-D-manno-octulosonic acid; β-D-DHA, 3-deoxy-D-lyxo-heptulosaric acid; β-L-AceA, β-L-aceric acid.

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Table 1. Composition of the experimental diets*

Groups…	Control	Pb-treated	Pb-treated + 2 g dRGII/kg diet	Pb-treated + 6 g dRGII/kg diet	Pb-treated + 18 g dRGII/kg diet
Diet ingredients (g/kg DM)					
Casein hydrolysate acid	200	200	200	200	200
Wheat starch	650	650	650	650	650
Maize oil	50	50	50	50	50
Alphacel, non-nutritive bulk	50	50	50	50	50
DL-Methionine	3	3	3	3	3
Choline bitartrate	2	2	2	2	2
AIN salt mix†	35	35	35	35	35
AIN vitamin mix‡	10	10	10	10	10
dRGII (g/kg diet)	0	0	2	6	18

dRGII, rhamnogalacturonan-II dimer.

* Powdered diet (100 g) was mixed daily with 100 ml deionized water to form a semi-liquid food prepared on site.

† AIN salt mixture (g/kg mixture): calcium phosphate dibasic 500; sodium chloride 74; potassium citrate monohydrate 220; potassium sulfate 52; magnesium oxide 24; manganese carbonate (43–48 % Mn) 3·5; ferric sulfate (16–17 % Fe) 6; zinc carbonate (70 % ZnO) 1·6; copper carbonate (53–55 % Cu) 0·3; potassium iodate 0·01; sodium selenite 0·01; chromium potassium sulfate 0·55; sucrose, finely powedered 118.

‡ AIN vitamin mixture (mg/kg mixture): thiamine hydrochloride 600; riboflavin 600; pyridoxine hydrochloride 700; nicotinic acid 3000; D-calcium pantothenate 1600; folic acid 200; D-biotin 20; cyanocobalamin (vitamin B_{12}) 1; retinyl palmitate (Vitamin A) pre-mix (75 mg) 1600; DL-α-tocopherol acetate (0·168 mg) 20000; cholecalciferol (vitamin D_3) (10 mg) 250; Menaquinone (vitamin K_2): 50; sucrose, finely powdered, 971·4.

metabolic cages, and intestinal mineral absorption was evaluated (balance study). During this period dietary feed consumption was recorded and all faecal and urinary excretions were collected for each animal. Rats were killed under sodium pentobarbital anaesthesia (40 mg/kg, intraperitoneally). Tibia, liver and kidney were then removed, rinsed and frozen at −20°C until analysis. Caecum and caecal contents were also sampled and weighed, and caecal content pH was determined.

Sample treatment and analysis

The faeces were freeze-dried and the wet and dry weights were determined. Urine volume was determined and 10 ml urine was sampled and acidified with 0·2 ml of 14 mol HNO_3/l. Adequate sub-samples of diet, faeces, urine, liver, one tibia and one kidney were dried overnight and then dry-ashed at 500°C for 10 h. The ash was dissolved in 0·5 ml of 14 mol HNO_3/l and 0·2 ml of 10 mol H_2O_2/l and heated at 110° for 2 h. The temperature was then increased to 130°C until the sample was dried. Then 5 ml of 0·14 mol HNO_3/l was added to every sample. In each case, an appropriate dilution with 0·14 mol HNO_3/l was performed before analysis. Pb concentrations were determined by inductively coupled plasma–mass spectrometry (ICP–MS). The mass spectrometer settings and plasma conditions were optimized with a solution of 10 μg In/l and the instrument operating conditions were as follows: radio frequency generator 27·12 MHz, forward radio frequency power 1350 W, reflected radio frequency power $<$3W, outer Ar flow rate 14 l/min, intermediate Ar flow rate 0·7 l/min, nebulizer Ar flow rate 0·76 l/min, mass resolution 0·9 Da, at 10 % of peak height. Data collection variables were as follows: total replicates per integration five, signal integration time per replicate 30 s, dwell time per sweep 20·4 s, scanning mode: peak hopping, five points per peak, sample uptake rate 0·6 ml/min.

Ca, Mg, Fe, Zn and Cu concentrations were determined by flame atomic absorption at wavelengths 422, 285, 248, 213·8 and 324·7 nm respectively using a Perkin Elmer 800 atomic absorption spectrometer (Perkin Elmer, St-Quentin en Yvelines, France).

Short-chain fatty acids were determined by GLC of portions of supernatant fractions of caecal contents (Demigné & Rémésy, 1985).

Calculations

Relative apparent absorptions of Pb, Ca, Mg, Fe, Zn and Cu were calculated using the following equation:

$$\text{Relative apparent absorption (\%)} = 100 \times ((\text{mineral intake} - \text{mineral faecal excretion})/(\text{mineral intake})).$$

Chemical mineral balance was determined as follows:

$$\text{Net balance} = \text{mineral intake} - (\text{faecal excretion} + \text{urinary excretion}).$$

The caecal short-chain fatty acids contents (μmol) were determined as caecal concentration (μmol/ml) × caecal water (g).

Statistical analysis

The data are expressed as group means and SE. ANOVA was used to test for any significant differences among experimental groups. If the *F* test was significant ($P<0·05$), the Student–Newman–Keuls multiple comparisons test was used to determine the specific differences between group means. Parametric ANOVA was used when the SD were homogeneous. If not, the non-parametric ANOVA Kruskall–Wallis test was used. If this last test indicated a significant difference among experimental groups ($P<0·05$), then the Mann–Whitney test was used to determine specific group differences.

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Results

Diet analysis indicated that the Pb levels of the control, and the Pb-treated rats were 0·11 and 2·92 mg Pb/kg diet. The target level was 3 mg Pb/kg diet in the Pb-treated groups. Food consumption and growth rate during Pb or dRGII treatments did not differ among the groups (Table 2).

Effect of rhamnogalacturonan-II dimer addition on caecal fermentation parameters

The addition of 2 or 6 g dRGII/kg diet did not influence caecal fermentation parameters, whereas the addition of 18 g dRGII in the diet resulted in an enlargement of the caecum corresponding to an increase in the caecal content (Table 3). The caecal enlargement was accompanied by a significant decrease in the caecal content pH (Table 3). In parallel, there was a significant increase in the short-chain fatty acids pool in rats receiving 18 g dRGII/kg, especially acetate and propionate (about a twofold increase; Table 3).

Effect of rhamnogalacturonan-II dimer addition on intestinal absorption and lead balance

The relative faecal excretion of ingested Pb was significantly increased in groups receiving dRGII after Pb treatment when compared with the control or the Pb-treated group (Table 4). Among the groups receiving dRGII, the highest percentage of relative faecal excretion of ingested Pb was observed for groups receiving 6 or 18 g dRGII/kg. dRGII ingestion was accompanied by a significant increase in Pb intake. Nevertheless, the absorbed amount of Pb was closely similar for all the groups (μg/d; Table 4). As expected, the previous Pb treatment resulted in a high urinary excretion in the Pb-treated group in comparison with the control group except for the group receiving 6 g dRGII/kg (Table 4). In spite of the observed changes in Pb faecal and urinary excretions, Pb balance was unchanged in animals receiving dRGII (Table 4).

Tissue retention of lead

As expected, Pb treatment led to high accumulation of Pb in tibia and kidney. Three weeks after stopping Pb treatment, bone Pb concentrations had spontaneously decreased by about 50 % for Pb-treated rats compared with the baseline (Fig. 2). The addition of dRGII did not significantly accelerate the decrease in tibia Pb concentrations at the three doses used in the present experiment. Similarly, 3 weeks after stopping Pb treatment, kidney Pb concentrations had spontaneously decreased by about 80 %. Only the addition of 6 g dRGII/kg was accompanied by a slight but significant decrease in kidney Pb concentration compared with the Pb-treated group (Fig. 2).

Intestinal absorption and tissue retention of magnesium, calcium, zinc, copper and iron

Neither Pb treatment nor dRGII addition had any significant effect on the absorbed amounts of certain essential minerals, which ranged from 155 to 195 mg/d, 3·7 to 4·6 mg/d, 354 to 465 μg/d, 114 to 135 μg/d, 19 to 31 μg/d, for Ca, Mg, Fe, Zn and Cu respectively. In addition, tissue concentrations of essential minerals and trace elements were determined and no significant changes were observed in the measured status indices (data not shown).

Discussion

In our previous work, a significant decrease in intestinal absorption and balance of Pb were observed when dRGII was given at 5 g/kg diet simultaneously with 3 mg Pb/kg diet (Tahiri *et al.* 2000). This observation suggests that dRGII probably acts by chelating both exogenous Pb (alimentary) and endogenous (biliary) Pb in the gastrointestinal tract, resulting in a decrease in their intestinal absorption. The low Pb balance suggested that dRGII might contribute to the detoxification of the body Pb. Our experiment was designed to test this hypothesis.

Here, we investigated whether dRGII would reduce the

Table 2. Effect of 3 weeks of lead treatment followed by 3 weeks of rhamnogalacturonan-II dimer (dRGII) addition on food consumption and growth rate*

(Means and standard errors for ten rats per group)

	Control		Pb-treated		Pb-treated + 2 g dRGII/kg diet		Pb-treated + 6 g dRGII/kg diet		Pb-treated + 18 g dRGII/kg diet	
Experimental diet…	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food consumption at day 0 (g/d)	17·46[a]	0·21	17·99[a]	0·35	17·77[a]	0·18	17·35[a]	0·23	18·51[a]	0·31
Food consumption at day 21, after Pb treatment (g/d)	19·97[a]	0·68	20·18[a]	0·84	20·58[a]	0·59	21·35[a]	1·12	20·98[a]	0·48
Food consumption at day 35 after dRGII treatment (g/d)	20·84[a]	0·62	24·09[a]	1·68	22·65[a]	1·08	22·52[a]	0·98	22·89[a]	0·82
Growth rate between day 0 and day 21 (g/d)	7·13[a]	0·24	7·32[a]	0·24	7·54[a]	0·25	7·21[a]	0·26	7·49[a]	0·17
Growth rate between day 21 and day 35 (g/d)	4·03[a]	0·26	4·26[a]	0·19	4·28[a]	0·22	4·18[a]	0·14	4·55[a]	0·25

[a] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 48–49.

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Table 3. Effect of 3 weeks of rhamnogalacturonan-II dimer (dRGII) addition on fermentation parameters*
(Means and standard errors for ten rats per group)

Experimental diet…	Control		Pb-treated		Pb-treated + 2 g dRGII/kg diet		Pb-treated + 6 g dRGII/kg diet		Pb-treated + 18 g dRGII/kg diet	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Caecal content (g)	2·27[a]	0·23	2·17[a]	0·13	2·20[a]	0·13	2·19[a]	0·16	2·82[b]	0·13
Caecal content pH	7·13[a]	0·04	7·29[b]	0·04	7·16[ab]	0·05	7·05[ab]	0·14	6·93[c]	0·06
Acetate (nmol/caecum)	127·3[a]	21·0	112·6[a]	9·3	110·3[a]	5·3	112·3[a]	8·4	184·6[b]	9·4
Propionate (nmol/caecum)	45·3[a]	8·2	38·5[a]	3·4	35·1[a]	1·4	38·0[a]	3·1	70·2[b]	4·1
Butyrate (nmol/caecum)	19·4[a]	2·1	20·8[a]	1·9	18·0[a]	1·1	19·8[a]	5·2	23·4[a]	3·0

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 48–49.

body burden of Pb in rats exposed for 3 weeks to Pb acetate in feed. We gave three different doses of dRGII for 3 weeks after the end of dietary Pb exposure. We started the Pb balance evaluation during the last week, when there was no more unabsorbed dietary Pb in the gastrointestinal tract, and the only Pb excreted in faeces, 2 weeks after the end of exposure, is Pb eliminated through the bile, which comes from the endogenous accumulation of Pb in the body.

The main result of the present study was that dRGII did not contribute to the detoxification of body Pb when given after chronic exposure to Pb. Intestinal absorption, balance and Pb tissue status remained similar at the end of the experiment in rats receiving the different doses of dRGII (0, 2, 6, 18 g/kg of diet). The hypothesis that dRGII may accelerate Pb detoxification was not confirmed in our conditions and study design. Even so, when dRGII was given concomitantly with Pb, the intestinal absorption was dramatically depressed (Tahiri *et al.* 2000). Hence dRGII seems to contribute mainly to detoxifying alimentary Pb (exogenous), but probably not that in body stores (endogenous).

Pectin binding of heavy metal ions has recently been evaluated in aqueous solution by Kartel *et al.* (1999). They showed that the pectins studied had very high binding affinities to Pb^{2+}. The action of pectins on heavy metal absorption has already been investigated. Most of the investigations on the action of pectin on Pb poisoning using animal models have shown a beneficial effect of pectin in Pb detoxification (Niculescu *et al.* 1968; Bondarev *et al.* 1979; Ivanov *et al.* 1997; Kushneva & Koltunova, 1997. However, Wapnir *et al.* (1980) failed to show any difference in intestinal Pb absorption or in urinary excretion of Pb after pectin feeding in rats. Other plant fibres, such as cellulose and glucomannan, have also been tested for their effect on Pb absorption in rats (Hayashi *et al.* 1991). The mechanisms involved in this effect are an increased Pb faecal and/or urinary excretion. According to Niculescu *et al.* (1968) and Macholz *et al.* (1989) both excretions are increased in rats and human subjects fed pectins. The increased plumburia in this type of experiment (pectin and Pb taken orally) suggests that pectin components may be absorbed and reach the blood to complex Pb, which will be excreted in the urine. The absorption of oligo-galacturonic acids has been shown to occur in rats after pectin feeding. According to Anger *et al.* (1994) when injected into the caecum of rats, 9 to 45 % oligogalacturonic acids are recovered in the urine within 16 h. This property is probably closely linked to the structure of the pectin considered. The data from our experiment showed that Pb urinary excretion was independent of the level of dRGII supplied in the diets. Therefore, dRGII cannot complex Pb in blood and promotes its elimination through urinary excretion. Consequently, dRGII seems to exert its effect mainly through an increased faecal excretion of the Pb present in the diet.

Table 4. Effect of rhamnogalacturonan-II dimer (dRGII) addition on the apparent intestinal absorption and chemical balance of lead in rats*
(Means and standard errors for ten rats per group)

Experimental diet…	Control		Pb-treated		Pb-treated + 2 g dRGII/kg diet		Pb-treated + 6 g dRGII/kg diet		Pb-treated + 18 g dRGII/kg diet	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food consumption (g/d)	17·84	0·75	19·93	0·34	18·29	0·74	18·95	0·34	18·27	0·51
Pb intake (μg/d)	2·00[a]	0·08	2·23[b]	0·04	3·01[c]	0·12	5·48[d]	0·10	12·33[e]	0·34
Faecal Pb excretion (μg/d)	1·17[a]	0·07	1·45[b]	0·05	2·43[c]	0·13	4·86[d]	0·14	11·42[e]	0·31
Relative faecal excretion of ingested Pb (%)	58·80[a]	2·34	65·29[a]	2·10	80·73[b]	3·10	88·77[c]	2·04	92·79[c]	1·92
Apparent absorption of Pb (%)	41·20[a]	2·34	34·71[a]	2·10	19·27[b]	3·10	11·23[c]	2·04	7·21[c]	1·92
Apparent absorption of Pb (μg/d)	0·83[a]	0·06	0·77[a]	0·05	0·58[a]	0·10	0·62[a]	0·12	0·91[a]	0·26
Urinary Pb excretion (μg/d)	0·030[a]	0·006	0·062[cb]	0·005	0·066[cb]	0·006	0·047[ab]	0·005	0·070[c]	0·008
Pb balance (μg/d)	0·764[a]	0·188	0·715[a]	0·169	0·516[a]	0·312	0·570[a]	0·362	0·841[a]	0·827

[a,b,c,d,e] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 48–49.

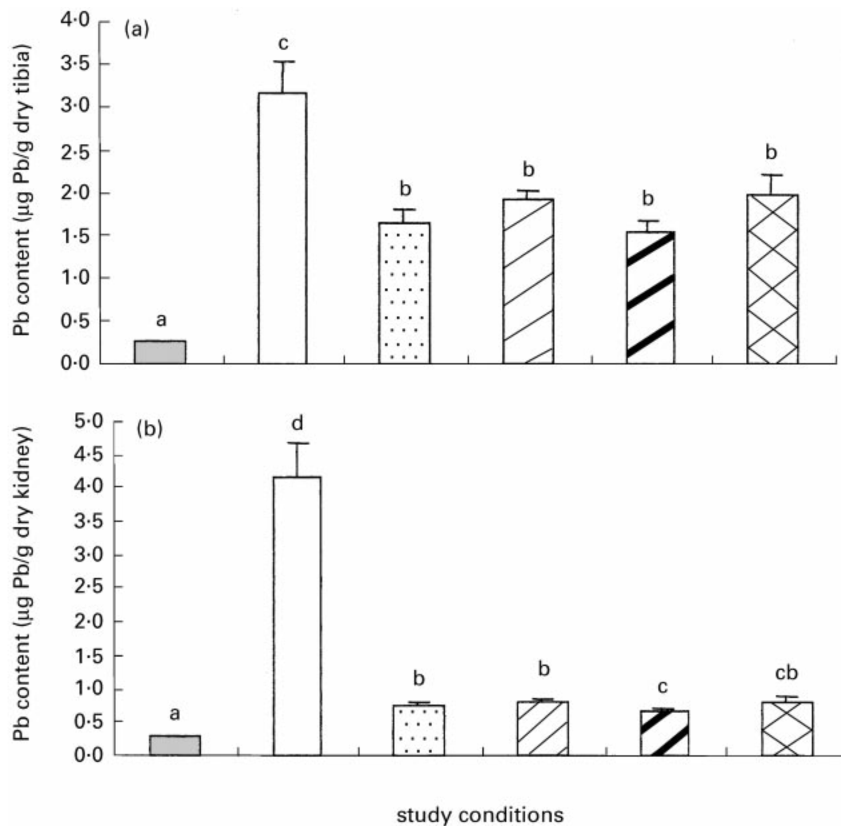

Fig. 2. Effect of lead administration followed by rhamnogalacturonan-II dimer (dRGII) administration on tibia (a) and kidney (b) lead levels in the rat. The control group (■) received a standard diet. Baseline (□) was a group receiving 3 mg lead/kg for 3 weeks after which animals were killed to serve as baseline for lead status. The lead-treated group (▧) received 3 mg lead/kg for 3 weeks and then a standard diet for 3 weeks. The three other groups received 3 mg lead/kg for 3 weeks and then 2(▨), 6(▨) or 18(⊠)g dRGII/kg for 3 weeks respectively. Values are group means for ten rats per group. Mean values for treatments with unlike letters were significantly different, $P<0·05$. Standard errors are represented by vertical bars.

RGII is a low molecular weight pectic polysaccharide (5000 D), present in the cell walls of growing plants, from which RGII can be released by an endo-α-1,4-polygalacturonase in a dimer from (O'Neill *et al.* 1990. dRGII has a strong ability to complex Pb (Ishii *et al.* 1999). Therefore, during the isolation procedure, the small amounts of Pb present in the environment may bind strongly to dRGII in the present study. The addition of dRGII to the animal diet was thus accompanied by a slight increase in Pb intake of about 30 µg/g dRGII added to the diet. However, when complexed to dRGII, Pb is not available for intestinal absorption. For the faecal excretion of Pb, the results of our study are consistent with our previous work (Tahiri *et al.* 2000). Quantities of absorbed Pb and balance were equivalent between animals receiving the supplemental Pb present in the added dRGII and controls whose diet was nearly devoid of Pb.

dRGII possesses a high capacity to bind certain minerals. The cations that bond to dRGII have some common properties: a valency of $^{2+}$ or $^{3+}$; a crystal ionic radius >0·95 Å; an electronic configuration with an incompletely filled sub-shell; a low energy of ionization; an affinity for O-donor ligands (O'Neill *et al.* 1996). Many metals, in particular the toxic metals Pb^{2+}, Ba^{2+}, Sr^{2+}, La^{3+}, Eu^{3+}, Ce^{3+}, Pr^{3+} and Nd^{3+}, fulfill these requirements and so are strongly chelated to dRGII. However, these characteristics of dRGII exclude the chelating of essential cations such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+} and Cu^{2+}. *In vitro*, the formation of dRGII from its monomer necessitates the presence of boric acid. The presence of an appropriate cation can also accelerate the formation of the dimer, which then strongly binds that cation, for example Pb. It is well known that dRGII, once formed, can bind added Pb, and that this depends on environmental conditions, e.g. pH. The optimal pH for this binding is between 3 and 5. We can speculate that dRGII can bind Pb in the gastric compartment where the gastric pH may be favourable. This can probably explain the observed inhibitory effect of dRGII on intestinal Pb absorption in our earlier work (Tahiri *et al.* 2000). On the contrary, it is possible that dRGII is unable to chelate Pb in the intestinal section where the pH is higher than 6. Bile Pb excretion is the main pathway for elimination of body Pb (Conrad & Barton, 1978; Alexander *et al.* 1986). It is well known that Pb undergoes enterohepatic re-circulation. In a previous study (M Tahiri, T Doco and C Coudray, unpublished results), we showed that the daily administration

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of 20 μg Pb intra-peritoneally in rats resulted in very high Pb excretion through the bile (more than two-thirds of the administered dose). Because of this, we expected dRGII to increase Pb excretion only if the Pb excreted through the bile could bind to dRGII in the intestine. It is highly likely that this Pb is not bound by dRGII in the upstream end of the intestine in the present study. This may at least partly explain the failure of dRGII to contribute to Pb elimination after chronic Pb exposure in rats.

The bioavailability of metals bound to fibres may depend on the ease of release of metals in the gut as well as on the extent to which the fibre is fermented in the colon (Ou *et al.* 1999). dRGII is a fermentable pectic polysaccharide that undergoes complete fermentation by the microflora in the large intestine. In fact, dRGII was not detectable in the rat faeces in the present study (data not shown). At the highest dose given in the present experiment (18 g dRGII/kg diet), the fermentation of dRGII results in a significant increase in short-chain fatty acid production in the rat caecum. This production was similar to that of inulin which is considered a well-fermented dietary fibre (data not shown). Fermentation may result in a dissociation and release, in the large intestine, of the small amount of Pb complexed to dRGII. Moreover, the short-chain fatty acid production resulted in a significant decrease in caecal pH, which in turn contributes to a better solubilization of Pb. This effect of fermentation is known to improve the adsorption of other minerals such as Ca and Mg (Demigné *et al.* 1995; Younes *et al.* 1996). A possible increase in intestinal absorption of Pb might thus occur through the para-cellular pathway in the colon when this fermentation is high. Pb is primarily absorbed in the small intestine and particularly in the duodenum and via the trans-cellular pathway (Diamond *et al.* 1998). Although the main site of intestinal absorption of Pb is located in the small intestine, an increased absorption in the lower parts of the intestine may increase the overall intestinal absorption of Pb. A negative effect of dRGII on Pb absorption is exerted since very small amounts of dRGII (0·5 g/kg diet), and a significant effect was observed for the dose 5 g dRGII/kg diet (Tahiri *et al.* 2000). In our experiment, we again observed a significant reduction effect on Pb kidney level at the dose 6 g dRGII/kg diet, but this effect disappeared at the dose 18 g dRGII/kg. It can be deduced that fermentation may be active at more than 10 g dRGII/kg, where the possible contribution of Pb absorption in the colon may be significant and should be taken into account.

In conclusion, our findings show that dietary Pb, when bound to dRGII, is essentially unavailable for intestinal absorption. Consequently, Pb present in the form of dRGII chelates in fruits, vegetables and fruit juices is probably not available for absorption. However, our results show also that the addition of dRGII after Pb exposure was not effective in assisting the process of Pb detoxification in rats. Therefore, although the presence of dRGII in food may have a preventive action on Pb absorption, it seems unable to act as a curative agent in Pb poisoning. Studies with human subjects are still needed to confirm these results in rats.

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