Dietary inulin intake and age can significantly affect absorption of the faecal marker dysprosium in rats

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It is believed that rare earth elements are not absorbed, and thus they are generally used in some mineral absorption studies as a faecal marker. The aim of the present study was to determine the effect of inulin intake and age on dysprosium (Dy) absorption in rats. Eighty male Wistar rats of four different ages (2, 5, 10 and 20 months) were randomised into either a control group or a group receiving 3·75 % inulin in their diet for 4 d and then 7·5 % inulin until the end of the study. The animals were fed fresh food and water *ad libitum* for 30 d. The intestinal absorption of Dy was determined from a 4 d (day 21 to day 25) balance study. Mean faecal Dy recovery (%) in the eight groups (3 months control, 3 months inulin, 6 months control, 6 months inulin, 11 months control, 11 months inulin, 21 months control, 21 months inulin) was 94·0 (SD 8·6), 64·8 (SD 10·1), 95·8 (SD 9·4), 81·5 (SD 12·1), 98·4 (SD 9·8), 87·8 (SD 9·5), 97·8 (SD 6·2) and 84·9 (SD 10·9), respectively. Our results showed clearly that dietary inulin intake decreased faecal Dy recovery in all four rat groups, and faecal Dy recovery was significantly higher in the old rats (10 and 20 months) than in the young and adult rats. These results show that the faecal recovery (or intestinal absorption) of Dy may vary greatly with nutritional or physiological states such as inulin intake or age. The use of rare earth elements as a faecal marker should be thus validated under each nutritional or physiological state before being employed in mineral absorption studies.

Intestinal absorption: Faecal marker: Dysprosium: Inulin: Fermentation: Age: Rat

The technique of faecal isotope balance or faecal isotopic monitoring employing the stable isotope approach is an important experimental tool for investigating the gastrointestinal absorption of minerals and trace elements (Mellon & Fairweather-Tait, 1997; Patterson & Veillon, 2001; Fairweather-Tait & Dainty, 2002). This requires a complete collection of stools for 3–4 d in rats and 5–10 d in adult man. In mineral absorption studies based on stable isotope approaches, the use of faecal markers allows researchers to control the faecal collection period or to reduce this period and determine mineral absorption in a small number of stools (Fairweather-Tait *et al.* 1997; Ulusoy & Whitley, 2000). This in turn avoids the need to carry out prolonged faecal collections and reduces the amount of stable isotope administered in these studies, which prevents the perturbation of mineral metabolism and reduces the cost of such studies.

In general, the faecal marker should not be absorbable, and its behaviour in the digestive tract should be identical to that of the mineral to be studied. Previous work has shown that the rare earth elements (REE) belonging to the lanthanide group are absorbed in negligible amounts in the mammalian gut (Durbin *et al.* 1956). They are therefore generally used in some macronutrient and micronutrient absorption studies as a faecal marker in animals (Coudray *et al.* 1998; Zhao *et al.* 2003; Jandacek *et al.* 2004) and man (Hutcheson *et al.* 1979; Schuette

et al. 1993, 2003; Fairweather-Tait *et al.* 1997; Harvey *et al.* 2002), and the use of many of REE has been validated by various investigators in normal conditions. The reliability of their use under particular nutritional conditions and physiological states, however, has not been validated.

There is now a growing interest in non-digestible inulin-type fructans in nutrition and health (Van Loo *et al.* 1999; Kaur & Gupta, 2002); these are fermented by the local microflora, stimulating the growth of bifidobacteria and lactobacilli, and it has been demonstrated that they increase the absorption of minerals, in particular Mg (Ohta *et al.* 1995; Coudray *et al.* 2003). Moreover, studies have shown that the ageing gastrointestinal tract becomes less efficient at absorbing some micronutrients, for example Ca, Zn and vitamins D and B_{12} (Saltzman & Russell, 1998). As the majority of investigators using REE as a faecal marker have employed dysprosium (Dy) in their studies, the present work was carried out to examine the validity of Dy as a faecal marker in these particular nutritional conditions and physiological states. With that aim, we determined the effect of dietary inulin intake and age on Dy recovery and absorption in rats aged 2–20 months. The work reported in this paper formed part of a larger study examining the effect of dietary inulin intake and ageing on the absorption of nutritional minerals (Ca, Mg, Zn, Cu) in rats (Coudray *et al.* 2005a,b).

Abbreviations: Dy, dysprosium; REE, rare earth element.
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Materials and methods

Materials and reagents

HNO_3 (ultrapure), Dy and indium standard solutions (1 g/l) were obtained from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available. Distilled water was used throughout. A Perkin-Elmer 6100DRC system (Perkin-Elmer Instruments, Courteboeuf, France) equipped with a Meinhard nebuliser (Perkin-Elmer) was used for Dy measurement.

Animals and diets

Eighty male Wistar rats aged 2, 5, 10 or 20 months were purchased from Janvier (Le Genest Saint Ile, France). Two groups were formed from each age bracket to receive either a control diet or a semi-purified diet containing inulin. The composition of these two diets is given in Table 1. Tested inulin (Raftaline) was purchased from Orafti (Tienen, Belgium). Powder diet (100 g) was made up with 100 ml distilled water to form a kind of semi-liquid food prepared on site each day. Dietary inulin level was maintained at 37·5 g/kg during the first 4 d and then at 75 g/kg from day 5 until the end of the experiment. The total duration of the experiment was 30 d. Food and water were given *ad libitum*. Food consumption and body weight were recorded weekly. Throughout the experiment, the rats were housed two per plastic cage (wire-bottomed to limit coprophagy) until the balance study and were then separated to one per metabolic cage from day 21 to day 30 in a temperature-controlled room (22°C) with a dark period from 20.00 hours to 08.00 hours. All procedures complied with the Institute's ethical guidelines on the care and use of laboratory animals.

Preparation of dysprosium solution and sample collection

$DyCl_3$ 250 mg was dissolved in 20 ml distilled water. The resulting solution was then topped up to 150 ml with distilled water and maintained for several days at +4°C until utilisation. The Dy content was checked before use. The rats were transferred to metabolic cages and housed individually 3 d before the beginning of the balance study to allow them to adapt to their new environment. Animals received approximately 1·7 ml Dy solution by gavage. The faeces of each rat were collected and quantified for four consecutive days (days 21–25), and excreted Dy in this medium and in the gavage solution was quantified by inductively coupled plasma/MS, as described later. The percentage faecal Dy recovery was calculated from 100 × (Dy excreted in the faeces/Administered Dy), and the percentage intestinal Dy absorption was calculated as follows: 100 × ((Administered Dy − Dy excreted in the faeces)/Administered Dy).

Analytical procedures

Faecal materials were dry-ashed (10 h at 500°C) and dissolved with concentrated HNO_3 and H_2O_2 on a heating plate until complete decoloration. The resulting solution was topped up to 10 ml with water and adequately diluted in 1 % HNO, and Dy concentration was determined by inductively coupled plasma/MS (6100DRC; Perkin-Elmer) using Dy as the external standard and indium as the internal standard. The instrument operating conditions were set as follows after optimisation with a solution of indium 1 μg/l: radio frequency power, 1050 W; nebuliser Ar flow rate, 0·79 l/min; auxiliary Ar flow rate, 1·2 l/min; outer Ar flow rate, 15 l/min. Data acquisition conditions were as follows: sweeps/reading 50; readings/replicate, 1; number of replicates, 3; dwell time, 100 ms; scanning mode, peak-hopping.

Data analysis

Values are given as means and standard deviations, and data were tested by two-way ANOVA using the General Linear Models procedure of the Super ANOVA package (Abacus, Berkeley, CA, USA). *Post-hoc* comparisons were performed using Fisher's least significant difference procedures. Differences of $P < 0.05$ were considered statistically significant.

Results

Food intake and growth rate

Inulin intake at the dose of 75 g/kg showed only a tendency for the animals to decrease their food intake in this study (Table 2). The slight decrease in food intake in inulin-fed rats led to a significant decrease in weight gain ($P < 0.05$) at the end of the experiment in inulin-fed rats compared with controls. The lower energetic value of the inulin diets (− 4 %) compared with the control diets may also be responsible for this reduced weight gain. In addition, food intake decreased significantly with increasing age, as expected (Table 2).

Faecal recovery and intestinal absorption of dysprosium

The gavaged amount of Dy of approximately 890 μg/rat was no different among the eight rat groups (Table 3). Faecal Dy levels averaged 81–137 μg/g faeces or 569–969 μg in the whole collected 4 d faeces pool. Faecal Dy excretion decreased significantly under inulin intake and increased significantly with age. Consequently, the percentage of faecal

Table 1. Dietary composition (g/kg) during the experiment

| | | Inulin | |
	Control	37·5 g/kg	75 g/kg
Wheat starch	650	612·5	575
Casein	200	200	200
Maize oil	50	50	50
Cellulose	50	50	50
Mineral mix (AIN 1993)*	35	35	35
Vitamin mix (AIN 1993)†	10	10	10
DL-Methionine	3	3	3
Choline bi-tartrate	2	2	2
Inulin	0	37·5	75

* Mineral mix AIN 1993 ensures the following mineral levels in the diet (mg/kg): Na, 1020; K, 3600; P, 4000; Ca, 5000; Mg, 500; Zn, 30; Fe, 35; Cu, 6; Mn, 54; Se,0·1; I, 0·2; Cr, 2.
† Vitamin mix AIN 1993 ensures the following vitamin levels in the diet: thiamin, 6 mg/kg; riboflavin, 6 mg/kg; pyridoxine, 7 mg/kg; nicotinic acid, 30 mg/kg; calcium pantothenate, 16 mg/kg; folic acid, 2 mg/kg; D-biotin, 0·2 mg/kg; cyanocobalamin, 10 μg/kg; vitamin K, 50 μg/kg; vitamin A, 4000 IU/kg; vitamin E, 50 IU/kg; vitamin D, 1000 IU/kg.

Table 2. Effects of inulin intake on weight gain and feed consumption during the experiment and during the faecal collection time in rats of different ages (3–21 months)

	3Mo C		3Mo I		6Mo C		6Mo I		11Mo C		11Mo I		21Mo C		21Mo I		Effect		
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	Inulin	Age	Interaction
Feed consumption (g/d)	23·1	3·2	22·6	3·7	21·3	3·3	23·9	2·5	18·5	2·5	19·2	2·1	19·0	1·7	16·3	2·8	NS	<0·0001	0·0282
Body weight, day 0 (g)	294	13	294	13	475	42	475	27	644	58	640	60	587	54	570	44	NS	<0·0001	NS
Body weight, day 30 (g)	480	28	464	40	597	64	592	47	701	63	692	67	662	76	610	39	NS	<0·0001	NS
Weight gain (g)	186	33	170	40	122	31	116	28	57	27	52	27	75	24	40	19	0·0208	<0·0001	NS

3Mo C, 3 months control; 3Mo I, 3 months inulin; 6Mo C, 6 months control; 6Mo I, 6 months inulin; 11Mo C, 11 months control; 11Mo I, 11 months inulin; 21Mo C, 21 months control; 21Mo I, 21 months inulin.
Data were tested by two-way ANOVA using the General Linear Models procedure of the Super ANOVA package (Abacus, Berkeley, CA, USA). *Post-hoc* comparisons were performed using Fisher's least significant difference procedures. Differences of $P < 0.05$ were considered statistically significant.

Table 3. Effects of inulin intake on faecal (dysprosium) Dy recovery and intestinal Dy absorption in rats of different ages (3–21 months)*

	3Mo C		3Mo I		6Mo C		6Mo I		11Mo C		11Mo I		21Mo C		21Mo I		Effect		
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	Inulin	Age	Interaction
Dy administered (µg)	902	25	878	11	887	8	890	11	883	9	893	14	885	13	896	12	NS	NS	0·0003
Faecal Dy (µg/g)	111·7	15·6	81·3	23·7	136·8	24·8	119·2	37·7	136·4	28·4	105·5	10·4	129·0	19·8	120·0	17·0	<0·0001	0·0002	NS
Faecal Dy (µg)	848	82	569	159	850	84	725	109	869	89	783	80	865	52	762	99	<0·0001	0·0014	0·0104
Dy recovery (%)	94·0	8·6	64·8	18·1	95·8	9·4	81·5	12·1	98·4	9·8	87·8	9·5	97·8	6·2	84·9	10·9	<0·0001	0·0010	0·0413
Dy absorption (µg)	54·3	77·7	309·6	160·2	37·3	83·3	164·3	107·2	14·0	86·4	110·0	86·2	19·5	54·7	134·9	97·6	<0·0001	0·0012	0·0529
Dy absorption (%)	6·0	8·6	35·2	18·1	4·2	9·4	18·5	12·1	1·6	9·8	12·3	9·5	2·2	6·2	15·1	10·9	<0·0001	0·0010	0·0413

3Mo C, 3 months control; 3Mo I, 3 months inulin; 6Mo C, 6 months control; 6Mo I, 6 months inulin; 11Mo C, 11 months control; 11Mo I, 11 months inulin; 21Mo C, 21 months control; 21Mo I, 21 months inulin.
* After Dy dosing, the faeces of each rat (ten animals per group) were collected and quantified for four consecutive days, and faecal isotope excretion was quantified by inductively coupled plasma/MS. Data were tested by two-way ANOVA using the General Linear Models procedure of the Super ANOVA package (Abacus, Berkeley, CA, USA). *Post-hoc* comparisons were performed using Fisher's least significant difference procedures. Differences of $P < 0.05$ were considered statistically significant.

Dy recuperation was significantly lower under inulin intake than in the control rats, and significantly higher in the old rats than in the young adult or adult rats. In parallel, net (μg) and relative (%) intestinal Dy absorption was significantly higher under inulin intake than in the control rats, and significantly lower in the aged rats than in the young adult or adult rats.

Discussion

The most commonly used faecal markers include colouring agents, polyethylene glycol, radio-opaque pellets and REE. Colouring agents such as carmine have been used as faecal markers to determine the time at which faecal collection can be started and stopped (Sian *et al.* 1996). Radio-opaque pellets, which can be recovered by physical extraction or identified using X-ray analysis, are used as faecal markers to ensure complete faecal collection in studies on man (Fairweather-Tait *et al.* 1997; Tahiri *et al.* 2001, 2003).

REE such as samarium, holmium, lanthanum, ytterbium and Dy are thought to be non-absorbable or absorbed in negligible amounts by the mammalian gut (Durbin *et al.* 1956) and have been traditionally used in animal experiments, particularly in determining the kinetics of digestion in ruminants (Crooker *et al.* 1982). They have also been used to determine nutrient absorption in some animal and human studies (Schuette *et al.* 1993; Fairweather-Tait *et al.* 1997; Coudray *et al.* 1998; Harvey *et al.* 2002; Matsui *et al.* 2002) in order to ensure complete faecal collection and reduce the faecal collection period. The first comprehensive use of REE as markers in mineral absorption studies was reported by Schuette *et al.* in 1993 in adult man. These authors observed a mean recovery of Dy of 104 % and a Dy excretion profile closely paralleling that of [70]Zn and [26]Mg. In contrast, Dy excretion did not correlate well with that of [65]Cu.

In another study, Fairweather-Tait *et al.* (1997) evaluated the usefulness of REE as non-absorbable faecal markers for Fe isotopes in absorption studies in man. They employed three different REE (samarium, ytterbium, Dy) in three studies using three different Fe isotopes ([54]Fe, [57]Fe, [58]Fe), reporting a mean recovery of samarium, ytterbium and Dy of 103 %, 98 % and 102 %, respectively. They also reported that Fe isotopes and REE had very similar faecal excretory patterns and showed that Fe absorption might be predicted from a 4 d faecal collection containing about an 80 % excretion of REE. Ulusoy & Whitley (2000) reported that excretion patterns of Fe isotopes and REE in human subjects were identical in only the first two or three faecal samples.

Finally in rat studies, Coudray *et al.* (1998) observed close faecal excretory patterns between faecal [67]Zn and [65]Cu and Dy. In addition, Matsui *et al.* (2002) reported that faecal Dy excretion closely paralleled that of [67]Zn, and that most of the Dy and [67]Zn was excreted within 3 d of administration.

Based on previous works and findings, we addressed the questions of whether the intestinal absorption of lanthanides, in particular Dy, might vary with the age of the animals, and of whether it might be influenced by dietary composition, such as by adding inulin to the diet. Our results showed clearly that the percentage recovery of Dy in the faeces was less than 100 % in the eight experimental groups. This percentage recovery was about 95 % in the young and adult and about 98 % in the old and very old rats receiving the control diet, and was only 65 % in the young, 81 % in the adult and about 86 % in the old and very old rats receiving inulin in their diet. The statistical analysis clearly showed that both inulin and age effects were significant in this study ($P < 0.0001$ and $P = 0.0010$, respectively). These results indicated that Dy might be partially absorbed in rats, and that this absorption might be modulated with dietary manipulation and varied with physiological states.

Ten Bruggencate *et al.* (2005) recently reported that dietary fructo-oligosaccharides increased intestinal permeability in rats. Inulin, used in our study, is a fructo-oligosaccharide that may increase intestinal permeability and thus increase Dy absorption. However, Sobotka *et al.* (1997) demonstrated that intestinal permeability, tested with ^{51}Cr-EDTA, was not influenced by inulin administration, added as dietary fibre into patients' fibre-free enteral nutrition. Indeed, the mechanisms by which inulin increases mineral absorption are not clear yet. It is known that inulin is metabolised by the bacteria in the large intestine, leading to an increased production of SCFA, mainly acetate, propionate and butyrate (Levrat *et al.* 1991; Campbell *et al.* 1997). SCFA production lowers the luminal pH, which increases mineral solubility and raises the mineral gradient between the luminal and serosal sides, thus allowing passive and active mineral transport to increase (Lopez *et al.* 1998; Scholz-Ahrens & Schrezenmeir, 2002; Coudray *et al.* 2003). In addition, SCFA, especially butyrate, serve as a fuel for mucosal cells and stimulate cell proliferation, which could in turn increase the absorptive surface area of the large intestine (Sakata, 1987; Lupton & Kurtz, 1993). These mechanisms may also be responsible for the increase in Dy absorption observed in this study.

Previous studies have already shown that some actinides and lanthanides can be absorbed by rats under particular conditions. Ulusoy & Whitley (2000) investigated the excretion profiles of REE and stable isotopes of Zn and Fe in man and reported a mean recovery of 94 % for the five REE, suggesting that some REE are absorbed much more than others. Recently, Matsui *et al.* (2002) reported that only about 90 % of administered Dy was recovered in the rat faeces collected over 5 d. Sullivan *et al.* (1986) reported that the absorption and retention of uranium, plutonium, americium and curium was substantially increased by fasting and by adding mild oxidising agents, and decreased by adding reducing agents. Moreover, they reported that the absorption of promethium, a lanthanide, was also increased by adding mild oxidising agents. In another study, Sullivan *et al.* (1984) examined the effect of age on lanthanide absorption by rats; they reported that the absorption of promethium and plutonium from the gastrointestinal tract was substantially higher for neonatal than for adult rats. This is in agreement with our results, in which Dy absorption was higher in the growing and adult rats than in the old rats.

In conclusion, our results showed clearly that if intestinal Dy absorption is negligible in normal conditions, it may vary largely under nutritional conditions (inulin intake) or physiological states (age). Thus, the validity of using REE as faecal markers should be determined for individual nutritional and physiological states. Studies are also needed to confirm these results in man.

Acknowledgements

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