Manganese absorption from mangold (*Beta vulgaris*): comparison of intrinsic and extrinsic labels

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The validity of the use of extrinsic labeling for studies of manganese absorption in man was evaluated using a test meal containing Beta vulgaris *intrinsically labeled with ^{54}Mn to which ^{52}Mn was added extrinsically. Whole body retention of the two isotopes was measured in a whole body counter during days 7–25 after administration of the test meal to six subjects. No difference in level of retention or rate of excretion was observed for the two isotopes. The calculated absorption (X ± SD) was 6.0 ± 3.4% and 6.2 ± 2.9% for ^{54}Mn and ^{52}Mn, respectively, resulting in a ^{54}Mn : ^{52}Mn ratio for fractional absorption from the test meal of 0.95 ± 0.14 (X ± SD). These results demonstrate that extrinsic labeling with radioisotopes of manganese is a valid method for measurement of manganese absorption from vegetables when studied in humans.*

Keywords: manganese; ^{54}Mn; ^{52}Mn; whole body counting; extrinsic label; intrinsic label

Introduction

The knowledge of manganese as an essential element for humans is very limited. The manganese requirement in humans is based on few data and is highly approximate. For adequate dietary recommendations for different ages, more information is needed on the absorption of manganese from different foods with identification of dietary factors influencing manganese absorption.

For elements with a low degree of absorption and for which the major excretory route is via the intestine, the chemical balance technique cannot be used to study the true absorption of the element. The optimal technique would therefore be to use biological incorporation of an isotope of the element into the food item (i.e., intrinsic labeling). However, the intrinsic labeling of foods with radioactive (or stable) isotopes is tedious and expensive and is therefore not practically useful when evaluating different foods in studies of mineral absorption in humans. The addition of an extrinsic inorganic label of the element to be studied is a far more easy and practical approach. When using this technique, isotope exchange is assumed to take place between the added isotope and the native element in the diet studied. However, the validity of this technique is a question that has been discussed extensively, and the technique should be evaluated for each element studied. Validation of the extrinsic labeling technique can only be achieved unequivocally by simultaneous administration of two different isotopes of the element studied to the same subject; one added as an intrinsic label (in vivo label) and another isotope added extrinsically (in vitro label). This technique has been used to validate the extrinsic labeling for iron using ^{59}Fe and ^{55}Fe[1,2] as well as for magnesium with a combination of radioactive (^{28}Mg) and stable (^{26}Mg) isotopes.[3]

Manganese is monoisotopic and so has no stable isotope tracer. Furthermore, ^{54}Mn is the only commercially available radioactive isotope of manganese. Therefore, to conduct investigations with the proposed study design is not possible without access to a non-commercial producer of another radiolabel of manganese. Our access to two radioisotopes of man-

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ganese and a very sensitive whole body counter with high spectral resolution provides a method for direct evaluation of the extrinsic labeling technique for studies of manganese absorption. We earlier reported an excellent agreement between the degree of retention and the rate of excretion of ^{54}Mn added intrinsically to chicken liver and ^{52}Mn added as an extrinsic label when studied in humans.[4] Even though vegetables are more important sources of manganese in the diet than animal products, many plant foods contain fiber, phytate, and other dietary factors potentially decreasing the bioavailability of manganese. Furthermore, the forms of manganese in animal and vegetable sources might be different, leading to a difference in the exchangeability between intrinsically and extrinsically added manganese. Therefore, we found it important to also validate the extrinsic labeling for a vegetable. In the present study, we have further confirmed the usefulness of the extrinsic labeling technique by using a leafy vegetable (*Beta vulgaris*) as the test meal, labeled both intrinsically and extrinsically and administered to humans.

Materials and methods

Subjects

Six healthy adults (2 men, 4 women) participated in the study. Mean age was 26 years (range 20–40). None of the volunteers had a history of gastrointestinal disorders. No medication or supplements of vitamins or minerals were taken by the subjects.

The subjects were given written and oral information about the aims and procedures of the study and informed consent was obtained from all subjects. The project was approved by the Ethical Committee, the Isotope Committee of Sahlgren's Hospital, Göteborg, and by the Human Subjects Committee at the University of California, Davis.

Experimental design

Measurements of body weight and height as well as background radioactivity were done before the study started. A fasting blood sample was drawn for determination of whole blood manganese and iron status indices.

The test meal was served in the morning after a 12 hr fast. No food or fluid was allowed during the next 3 hr after intake of the labeled test meal, but after this time the volunteers consumed their normal diet.

Whole body retention measurements were performed on day 7 after administration and thereafter 2–3 times weekly during the following 3 weeks.

Test meal

Intrinsically labeled mangold (*B. vulgaris*) was prepared by growing the plants from seedling stage in pots to which ^{54}Mn was added to the soil in portions of 0.3 MBq when the seeds were planted, and thereafter once a week during 6 weeks. The pots were kept indoors in a laboratory and placed close to a window. The plants were harvested after approximately 3 months, rinsed in deionized distilled water, and heated in a microwave oven using a minimum of water. The individual portions were kept frozen until served. Two batches of *B. vulgaris* were prepared at two different times for practical reasons. The first batch (25 g/portion) was administered to subjects 1 and 2, while a second batch (30 g/portion) was served to subjects 3–6.

Radioactivity in each portion was measured separately in the whole body counter before serving. The mean content of ^{54}Mn in each portion was 0.067 MBq (range 0.035–0.114 MBq). Labeled *B. vulgaris* was served together with a bread roll made of 30 g refined wheat flour, 10 g butter, and 200 g deionized distilled water.

Analyses

Content of manganese in freeze-dried aliquots of the two samples of *B. vulgaris* and the bread roll was analyzed by atomic absorption spectrophotometry (Perkin Elmer Model 360) after dry ashing (450° C) while manganese content of the ^{52}Mn solution was analyzed by electrothermal atomic absorption (Perkin Elmer Zeeman/3030), equipped with graphite furnace HGA 600 (Perkin Elmer, Überlingen, Germany). A reference standard material for manganese (Bovine liver 1577a, National Bureau of Standards, USA) was run simultaneously and tell within the certified range. Whole blood manganese was analyzed by electrothermal atomic absorption[5]; (Perkin Elmer Zeeman/3030) with the use of magnesium-nitrate as a matrix modifier.[6]

^{54}Mn ($^{54}MnCl_2$) was purchased from New England Nuclear (Boston, MA, USA) while ^{52}Mn was produced especially for this study in the cyclotrone at the Department of Physics, University of Oslo (Oslo, Norway).[4] The ^{52}Mn solution (100–200 μl) was pipetted on the heated individual portions of *B. vulgaris* and mixed with the vegetable just before serving. The mean content of ^{52}Mn added to each portion was 0.240 MBq (range 0.115–0.516 MBq). The test meal had a total manganese content of 390 and 1065 μg in the two administrations; 265 and 940 μg from the *B. vulgaris*, and 125 μg from the bread, respectively. The addition of ^{52}Mn added 3–6 μg cold manganese to each portion.

The whole body counter laboratory is equipped with a double chamber iron room with 150 mm thick walls containing two different detector systems.[7] Detector system 1, which was used in this study, consists of two 130 × 100 mm NaI (Tl) detectors mounted on opposite sides of a patient couch and attached to a motor-driven X-Y scanning system. This detector system enables studies of more than one isotope simultaneously present in the body; in this case to measure whole body retention of ^{54}Mn and ^{52}Mn.[4] Manganese absorption and turnover rate were calculated according to the method we have described earlier.[8]

The resulting radiation exposure was evaluated by using the model of ICRP[9,10] which gives the exposure of the whole body after ingestion of a certain activity.

Table 1 Whole blood manganese (B-Mn) and iron status indices of participating subjects

Subject	Sex	B-Mn (nmol/L)	Hemoglobin (g/L)	Serum Fe (μmol/L)	TIBC (μmol/L)
1	M	160	138	18	60
2	F	90	118	21	58
3	F	230	129	14	61
4	M	270	155	17	57
5	F	290	137	16	44
6	F	210	139	18	55

Table 2 Fractional manganese absorption of ^{54}Mn (intrinsic label) and ^{52}Mn (extrinsic label), and turnover rate during days 7–25 of ^{54}Mn and ^{52}Mn for the six subjects, respectively

Subject	Absorption (%)		Ratio absorption	Turnover rate (days)		Ratio turnover rate
	^{54}Mn	^{52}Mn	^{54}Mn : ^{52}Mn	^{54}Mn	^{52}Mn	^{54}Mn : ^{52}Mn
1	11.8	10.2	1.2	10.4	13.6	0.76
2	3.0	3.3	0.91	27.7	27.0	1.03
3	7.0	8.5	0.82	11.5	11.7	0.98
4	4.3	5.2	0.83	10.9	11.8	0.92
5	2.8	3.0	0.93	18.6	23.5	0.80
6	6.9	7.0	0.99	11.4	13.0	0.88
X	6.0	6.2	0.95	15.1	16.8	0.90
SD	3.4	2.9	0.14	6.9	6.7	0.10

In this study, the radiation exposure was in the range 0.2–0.9 mSv to each subject.

Statistics

Student *t* test for paired samples was used.

Results

All values for manganese in whole blood and iron status indices fell within the normal range for our laboratory except for one of the women (subject 2) who had a whole blood manganese level of 90 nmol/L (reference values for women: 135–330 nmol/L) (*Table 1*).

The whole body retention measurements for each subject are shown in *Figure 1*. The mean absorption (X ± SD) was 6.0 ± 3.4% and 6.2 ± 2.9%, range 2.8–11.8% and 3.0–10.2% for ^{54}Mn and ^{52}Mn, respectively. Mean (X ± SD) turnover rate measured during days 7–25 was 15.1 ± 6.9 days (range 10.4–27.7 days) and 16.8 ± 6.7 days (range 11.7–27.0 days) for ^{54}Mn and ^{52}Mn, respectively, *Table 2*. The ^{54}Mn : ^{52}Mn ratio for fractional absorption and turnover rate was 0.95 ± 0.14 day and 0.90 ± 0.10 (X ± SD), respectively. No significant differences were observed between ^{54}Mn (intrinsic label) and ^{52}Mn (extrinsic label) for fractional absorption or turnover rate, respectively.

Discussion

Whether retention of an extrinsic label added to a meal can be assumed to reflect the absorption of the intrinsic mineral of the meal is a crucial question with important implications when designing studies of mineral bioavailability. If only intrinsic labeling can be used,

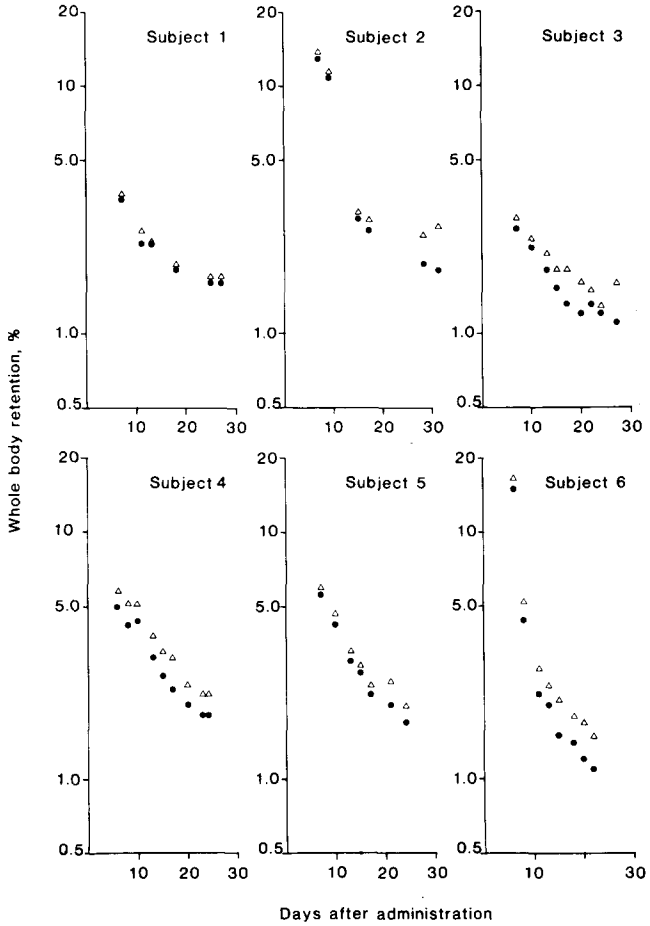

Figure 1 Whole body retention of ^{54}Mn (intrinsic label) (●) and ^{52}Mn (extrinsic label) (△) in the six subjects, respectively.

a very small number of studies can presumably be carried out, using very selected food items. However, if it is possible to conclusively demonstrate that an intrinsic and an extrinsic label are absorbed to the same extent, the experimental procedure for preparation of test meals can be simplified considerably.

Simultaneous and thereby direct comparison of the absorption of an intrinsic and an extrinsic radioactive label of manganese was possible in this study since another isotope besides ^{54}Mn was available (i.e., ^{52}Mn). When using only one radioisotope as the tracer added both intrinsically and extrinsically, the labeled food items must be administered on two separate occasions with a time lapse of at least one month,[8] or be administered to two different groups of subjects. This study design precludes a direct and more precise way of evaluating the labeling technique. With simultaneous administration of the intrinsic and extrinsic label, the potential influence of variation in mineral absorption due to changes in, for example, mineral status or dietary intake is eliminated. These factors have not yet been evaluated in detail with regard to influence on absorption of manganese.

Very few studies have been reported using the same approach as this study, due to the practical problems involved. The lack of more than one radioisotope has led to studies of zinc absorption in which ^{65}Zn added extrinsically was compared to ^{65}Zn intrinsically incorporated into beef by repeated administrations to adults,[11] while copper absorption has been studied from an extrinsic and intrinsic stable isotope (^{65}Cu) added to different foods and using repeated administrations to women.[12] These studies suggest that measurements of zinc and copper absorption from the studied food items using extrinsic tracers are valid.

In an earlier study, we showed that extrinsic labeling of manganese is feasible for chicken liver[4] and the present study was conducted to show that the labeling technique is useful for a vegetable food item as well. The total content of manganese in the two test meals varied with a factor of approximately 3 due to different levels of manganese in the two batches of *B. vulgaris*. The difference in manganese content is probably due to differences in the proportions of leaves to stems included in the prepared batches as well as differences in the conditions under which the plants were grown (i.e., differences in the composition of the soil). However, we have previously shown only a marginal effect of isotope dilution on fractional absorption of manganese in humans[13] and the difference in manganese content is therefore not considered as being a problem in this study.

The mean manganese absorption demonstrated in this study is similar to our earlier observation of mean manganese absorption from infant formula with high bioavailability, 5.9%.[14] The often quoted figure for manganese absorption in humans; 3 ± 0.5%[15] would therefore seem to be an underestimate of the fractional manganese absorption from certain foods. Based on these results, mangold seems to be a good source of manganese with high bioavailability.

In conclusion, absorption of intrinsic (^{54}Mn) and extrinsic (^{52}Mn) tracers from a test meal based on *B. vulgaris* did not differ significantly when studied in six adults. The results from this study confirm that the extrinsic labeling technique using a radioactive isotope of manganese is valid when used in studies of humans.

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