Absorption of calcium from a leafy vegetable rich in oxalates

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1. Plant foods, especially green leafy vegetables, are the cheapest and richest source of calcium in developing countries and are recommended in balanced diets. Hence it was worth while obtaining further information about the availability of Ca from these commonly-consumed green leaves.

2. The green leafy vegetable belonging to *Amaranthus* spp. was taken as a suitable experimental model because it is not only commonly-consumed, but is also rich in Ca and oxalates. In the first experiment, each of the eight adult male subjects was given a basal diet containing negligible Ca, a milk diet and an '*Amaranthus*' diet, both containing the same amount of Ca. These diets were given on 3 consecutive days and the urinary Ca excreted in a 6 h period after the test diet was considered an approximate index of Ca absorbed in that period. The second experiment was designed to study the influence of *Amaranthus* spp. on the availability of milk Ca. Each of the ten adult male subjects were given a basal diet, a milk diet and a diet containing the same amount of milk with *Amaranthus* spp. added to it. Urinary Ca excreted in a period of 6 h was estimated.

3. While availability of Ca from milk was good, that from *Amaranthus* leaves was low. The ingestion of *Amaranthus* leaves together with milk adversely affected the absorption of milk Ca.

4. In view of these findings, recommendation of green leaves as a regular source of Ca to the vulnerable section of the Indian Community should be considered with caution. Since green leaves are a main source of other nutrients, especially β-carotene, their consumption cannot be discouraged and hence leaves low in oxalates should be recommended.

Except for a small proportion of people belonging to the high socio-economic group, the majority of the population in India, obtain their nutritional requirements from plant foods such as cereals, pulses and vegetables. The intake of foods of animal origin such as milk products, eggs and fish is very small, because they are expensive commodities. Many of the vegetables are poor in most of the nutrients except ascorbic acid, but green leaves are exceptions because they are rich sources of several nutrients such as calcium, iron and β-carotene. The intake of green leaves, however, is low in many areas in India (Gopalan, Balasubramanian, Ramasastri & Visweswara Rao, 1969) except during certain seasons. It was found by Pingle (1975) that during the monsoon period in some tribal groups of Central India, 20–50% of the total intake of Ca was from green leaves showing that these vegetables can be suggested as good sources of Ca.

A number of the commonly-consumed green leafy vegetables are known to be rich in oxalic acid (Kohman, 1939; Rau & Murthy, 1942; Srivastava & Krishnan, 1959; Singh, 1973; B. V. Ramasastri, unpublished results). Numerous studies carried out on laboratory animals have shown that Ca from green leaves, such as spinach (*Spinacia oleracea*) which is rich in oxalic acid, is not utilized (Fincke & Sherman, 1935; Kohman, 1939; Speirs, 1939). However, studies on human subjects are few and the results contradictory (McLaughlin, 1927; Bonner, Hummel, Bates, Horton, Hunscher & Macy, 1938; Basu & Ghosh, 1943; Johnston, McMillan & Falconer, 1952; Walker, Walker & Wadvalla, 1975). The FAO/WHO Expert Group on Calcium Requirements (WHO, 1962) decided not to give serious consideration to the effect of these interfering substances on the utilization of Ca. This may be due perhaps to the lack of adequate information on this subject, especially in human situations. The Expert Group of the Indian Council of Medical Research based on the FAO/WHO Report, recommended in 1968, that green leaves should be included in balanced diets, as sources of many nutrients, especially for vulnerable segments of the population such as growing children and pregnant and lactating women (Gopalan & Narasinga Rao, 1968). For these reasons, it was considered worth while obtaining further information about availability of Ca from commonly-consumed green leafy vegetables. The simple experimental approach suggested by Walker *et al.* (1975) to determine availability of Ca was used in this study and the results obtained are reported in this communication.

MATERIALS AND METHODS

Subjects and experimental procedures

Healthy male subjects aged between 20 and 35 years and belonging to the scientific staff of the National Institute of Nutrition, Hyderabad were selected for the study, and the nature and importance of the work explained to them.

Edible leaves of the *Amaranthus* spp. (mixture of *Amaranthus gangeticus*, *Amaranthus gracilis* etc.) marketed as Totakoora were used as an experimental model in the study, not only because they are commonly-consumed by the urban and rural population but also because they are rich both in Ca and in oxalic acid.

The first part of the study (Expt 1) was designed to determine the availability of Ca separately from *Amaranthus* leaves and from milk, while the second part of the study (Expt 2) was designed to determine the availability of Ca from milk when given together with *Amaranthus* spp. In Expt 1, eight subjects were selected and each subject was given each of the three dietary regimens; a basal low-Ca diet, a diet consisting only of *Amaranthus* leaves and a milk diet. The diets were given on different days, ensuring that the period between each of the three diets was not more than 3 d. This was mainly to minimize the possible day-to-day variation in the urinary excretion of Ca.

The basal diet consisted of sago prepared from tapioca starch (*Manihot esculenta*) which contains Ca in very small amounts (Gopalan, Ramasastri & Balasubramanian, 1971). The amount of Ca in *Amaranthus* leaves and in the milk diets was approximately 450 mg. The total oxalate content (as anhydrous oxalic acid) of the *Amaranthus* leaves used in the experiment ranged from 7·4 to 10·63 g/kg and 50% of the oxalate was found to be in the form of water-soluble oxalates.

The initial diet given to the subjects consisted of *Amaranthus* leaves. After these leaves had been analysed for Ca content as quickly as possible, the quantity of milk containing an equivalent amount of Ca was given in the subsequent diet to the subject. The basal diet was given either after the *Amaranthus* diet or after the milk diet.

In Expt 2, ten subjects were selected and each of them was put on a 3 d dietary regimen of the basal diet, milk diet or milk plus *Amaranthus* diet, in a random order. The amount of Ca in the milk was approximately 430 mg, that in 100 g *Amaranthus* leaves which was given together with the milk approximately 350 mg Ca.

The experimental diets were given at 07.00–08.00 hours, after overnight fasting. The subjects were asked to empty their bladders just before the experiment and urine was then collected for a 6 h period. Sago fried with a little seasoning was supplied to the subjects 3–4 h after the experimental diet was given. The total amount of energy given in the diet to each subject during the period of the experiment was approximately 1672 kJ (400 kcal). The green leaves were cut into small pieces and then cooked in a pressure cooker for 5 min. The water from the leaves was not thrown out, thus ensuring that there was no loss of Ca or oxalates during the cooking. To make the diet palatable, the leaves were fried with small amounts of seasoning containing a negligible quantity of Ca. During the experiment, the subjects were allowed to drink water whenever they were thirsty.

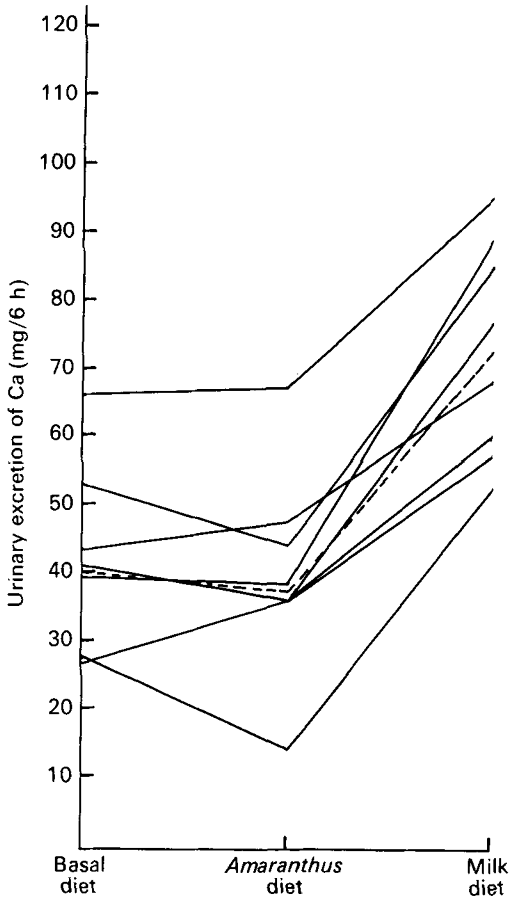

Fig. 1. Urinary excretion of calcium by adult male subjects given a basal diet, a diet containing *Amaranthus* spp. leaves and a milk diet (for details of diets, see p. 120). (------), Median values.

Analytical methods

The oxalic acid content (both total and soluble) of the *Amaranthus* leaves was estimated by the method of Baker (1952) and the leaves were analysed for Ca content by the micro-method described by Association of Official Agricultural Chemists (1965) for plant foods. The milk samples were analysed for Ca by the complexometric titration method described by Baron & Bell (1959).

The urinary Ca was estimated by the complexometric method of Baron & Bell (1959), but with a modification involving 'back' titration with a standard Ca solution after the addition of a known amount of excess EDTA (Kamal, 1960). This was done because in our experience, in the analysis of urine, there was a sharper end-point by 'back' titration than by 'direct' titration with EDTA. The details of the modified procedure are as follows.

After separation of phosphate from 2 ml urine as described by Baron & Bell (1959), 1 ml of the supernatant fraction was taken in a test-tube and 0·5–1 mg calcein-thymolphthalein indicator was added to it. The mixture was adjusted to pH 12 by drop-wise addition of approximately 4 M-sodium hydroxide until the colour just changed to mauve. EDTA solution (0·93 g disodium salt/l water; 2 ml) and 5 ml glycine buffer (pH 12·7) were then added and the mixture titrated slowly with a standard calcium carbonate solution (100 μg

Table 1. *Expts 1 and 2†. Urinary excretion of calcium from a low-Ca basal diet, a diet containing Amaranthus spp. leaves, a milk diet and a diet containing milk and Amaranthus spp. leaves by adult male subjects*

(Mean values with their standard errors for eight subjects in Expt 1 and ten subjects in Expt 2)

	Urinary Ca excretion (mg Ca/6 h)			
	Basal diet	Milk diet	*Amaranthus* diet	*Amaranthus* basal
Expt 1	42·16 ± 4·519	72·206 ± 5·534	39·856 ± 5·221	−2·307 ± 2·617NS
Expt 2	39·496 ± 4·373	80·795 ± 8·563	—	—
	Milk-basal diet	Milk + *Amaranthus* diet	Milk + *Amaranthus*-basal diet	Milk- (Milk + *Amaranthus*) diet
Expt 1	31·04 ± 3·167***	—	—	—
Expt 2	41·22 ± 5·388***	50·315 ± 5·151	10·819 ± 3·740*	30·48 ± 4·591***

NS, not significant.
Values were statistically significantly different: $*P<0·05$, $***P<0·001$.
† For details of experimental procedures, see p. 120.

Ca/ml), shaking the tube well after the addition of each drop of solution until green fluorescence just appeared and remained permanent.

Calculations of results

The equation used to calculate the urinary Ca content was:

$$\text{Ca content (mg) of urine} = (B-T)\times\frac{5}{2}\times\frac{100}{1000}\times\text{volume of urine},$$

where B (blank) is the volume of standard Ca solution required to titrate 2 ml EDTA solution to which were added 1 ml of water, 5 ml glycine buffer and 0·5–1 mg indicator. T (test) is the titre value of the urine supernatant fraction, and the factor $\frac{5}{2}$ corresponds to dilution of 2 ml urine for the separation of phosphates as described by Baron & Bell (1959). All the analyses were done in duplicate.

RESULTS

The results of Expt 1 are given in Fig. 1 and in Table 1. It can be seen that on all three diets there were individual differences in the urinary excretion of Ca in the 6 h period, but the mean increase in the excretion compared with the basal level was not significantly more when the source of Ca was from *Amaranthus* leaves (paired t test). With milk as the source of Ca, however, the increase in excretion was significantly ($P<0·001$) higher thus showing that while the availability of Ca from milk was good, it was very low from *Amaranthus* leaves.

The results of Expt 2 are given in Fig. 2 and Table 1. As in Expt 1 the mean increase in the urinary excretion of Ca compared with the basal level was significantly higher ($P<0·001$) when milk was the main source of Ca. However, the addition of *Amaranthus* leaves to this milk diet resulted in a significant decrease ($P<0·001$) in urinary Ca excretion.

The median values given in Figs 1 and 2 do not show a significant departure from the mean values indicating clearly a Gaussian distribution.

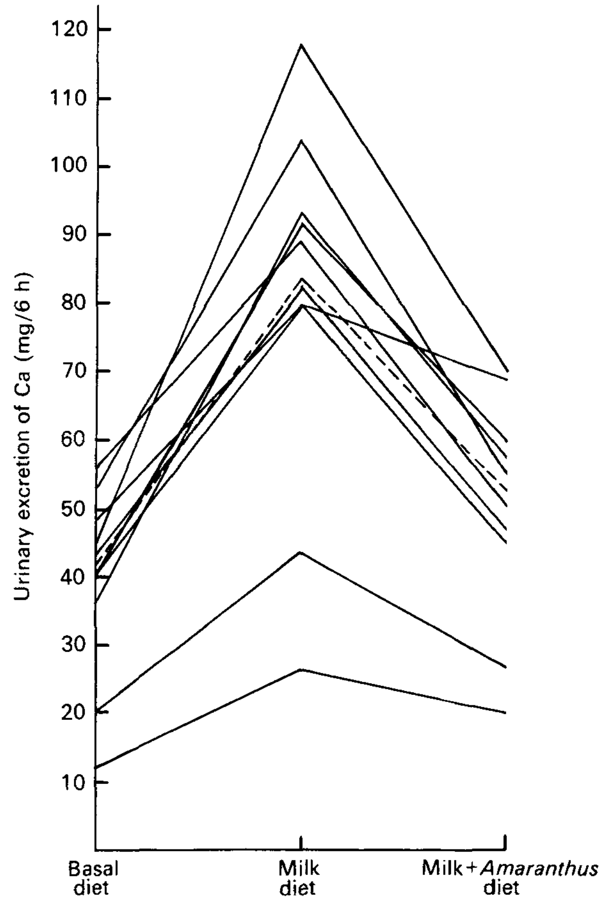

Fig. 2. Urinary excretion of calcium by adult male subjects given a basal diet, a milk diet and a diet containing milk and *Amaranthus* spp. leaves (for details of diets, see p. 120). (------), Median values.

DISCUSSION

Observations by McCance & Widdowson (1942) and Knapp (1947) demonstrated that an increase in Ca intake is followed by an increase in the amount excreted in the urine, indicating that urinary Ca excretion is an index of Ca absorbed. However, these observations were based on long-term balance studies in which many factors influenced the utilization of Ca from a particular foodstuff. For studying the absorption of Ca from a single dietary source it is more appropriate if the investigation is carried out over a short period of time. This type of experimental approach was first used by Walker *et al.* (1975), where the authors took the Ca excreted in the urine in a 6 h period after consumption of a diet as an approximate index of the amount of Ca absorbed within that period. This was based on findings of Szymendera, Heaney & Saville (1972), who, using a technique developed for measuring absorption of Ca by administering two isotopes of Ca orally and intravenously to human subjects, found that Ca absorption occurs maximally in the duodenum and proximal jejunum and that it was essentially complete within 4 h. Although the rates of absorption of Ca from different foods may be variable, a period of 6 h can be considered sufficient for maximal absorption of Ca to have occurred from the two types of food used in this study.

The results of experiments reported here clearly show that Ca in *Amaranthus* leaves is poorly absorbed and that when the leaves were given along with milk, they rendered the milk Ca less available for absorption. The two main substances which are known to interfere with Ca availability from plant foods are phytates and oxalates. Since green leaves do not contain phytates (Giri, 1938; Sundarajan, 1938; McCance & Widdowson, 1960) the probable reason for the poor absorption of Ca from *Amaranthus* leaves is the high oxalate content. The results obtained in the present study confirm earlier findings of others that Ca availability is low in the presence of high amounts of oxalates (Bendana-Brown & Lim, 1957).

The observation that ingestion of *Amaranthus* leaves together with milk adversely affected the absorption of milk Ca in man has obvious public health importance. This finding is similar to those reported by other workers using rats (Speirs, 1939; Rau & Murthy, 1940; Costa & Taccola, 1943). Kohman (1939) has also shown that in rats given a diet consisting of spinach and other vegetables the Ca was not utilized and that the oxalates present in the leaves rendered the Ca from the rest of the diet unavailable.

In view of these findings in man, the recommendation that leafy vegetables be included in diets as a source of Ca for growing children and pregnant and lactating mothers may need revision, particularly when such vegetables are rich in oxalates as well. Experimental studies on rats by Kohman (1939) have demonstrated that oxalic acid in spinach prejudices the calcification of bone and increases the mortality rate, whereas leaves with negligible oxalate content produce excellent animals that deposit four times as much Ca on a per unit body-weight basis, as compared to those receiving spinach. In most of the commonly-consumed leaves, the ratio, Ca:anhydrous oxalic acid is less than 0·5 (B. V. Ramasastri, unpublished results), indicating that all the Ca is bound to oxalate in the leaves and is unavailable for absorption. Since the intake of leafy vegetables cannot be discouraged as they are the only source of nutrients such as β-carotene and to some extent Fe in the majority of Indian diets, leaves having low oxalate contents should be recommended, particularly in view of the findings of Speirs (1939), Kohman (1939), Rau & Murthy (1942), Oke (1969) that in general, the Ca of leaves containing small amounts of oxalates is better absorbed.

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