The effects of supplements varying in carotene and calcium content on the physical, biochemical and skeletal status of preschool children

By R. RAJALAKSHMI, S. S. SAIL, D. G. SHAH AND S. K. AMBADY

Department of Biochemistry, Faculty of Science, M.S. University of Baroda, India

(*Received* 19 *July* 1972 – *Accepted* 28 *October* 1972)

1. Studies were made of the effects of food supplements varying in carotene and calcium content on the nutritional status of children aged 2–6 years. Three groups of children who were given a dietary supplement for 6 months and a fourth group of controls were investigated. The supplement was the fermented food dhokla, made of wheat and Bengal gram (*Cicer arietinum* L.) (group 1) or dhokla with added greens (group 2) or dhokla with added greens (fenugreek) and lime powder (a mixture of CaO, $Ca(OH)_2$ and $CaCO_3$) (group 3).

2. Studies were made of the effects of the above supplements on height, weight, skeletal status as judged by increments in bone age and cortical thickness of the second metacarpal bone and femur, and biochemical status as judged by the composition of blood (haemoglobin), serum (protein, albumin and vitamin A) and urine (creatinine, nitrogen, thiamin, riboflavin and vitamin C).

3. All the groups given the supplement were found to be superior to the controls as judged by these criteria. The size of the increment generally increased from group 1 to group 3 and the differences were most marked for bone age and cortical thickness.

4. The results suggested that (*a*) calcium deficiency is a crucial factor in the aetiology of skeletal retardation in young children and (*b*) that at the levels of calcium (560 mg) and phosphorus (980 mg) consumed, and with liberal exposure to sunlight, a dietary supply of vitamin D may not be a crucial factor for skeletal development in young children.

It is well known that in developing countries children in the post-weaning period are subject to undernutrition and malnutrition. Improving the nutritional status of young children by food supplementation or enrichment is engaging the attention of both nutritionists and administrators. In this laboratory the approach has been to use cheap locally available foods and simple cooking procedures for the alleviation of malnutrition and undernutrition in this group (Rajalakshmi & Ramakrishnan, 1967).

The diets of children in India and elsewhere are particularly deficient in energy, protein, vitamin A, calcium and iron. In previous studies in this laboratory attempts were made to correct these deficiencies and to effect the nutritional improvement of this age group by the administration of a breakfast gruel made of wheat, Bengal gram (*Cicer arientinum* L.) and groundnut (*Arachis hypogaea* L.) and a lunch consisting of leafy and other vegetables and a fermented preparation (dhokla) made of wheat and Bengal gram incorporated with lime. Children given this diet were found to show significant improvement in nutritional status as judged by increments in height and weight and by clinical, biochemical and radiological examination (Ramachandran, 1968). As it is not always feasible to organize both breakfast and lunch as part of a feeding programme and as one-dish meals are more conveniently administered, we tried to investigate whether a supplement of just one dish containing all the critical nutrients would be effective in improving the nutritional status of children.

Further, in the earlier studies, a considerable amount of skeletal retardation was found in young children. Skeletal status was found to be greatly improved with the breakfast and lunch given, but this could have been due either to an increase in energy and protein intakes provided by wheat, Bengal gram and groundnut or to the calcium and vitamin A provided by leafy vegetables or to the additional calcium incorporated in the form of lime in fermented dhokla. The present investigations were undertaken to study the comparative improvement in nutritional status brought about by a supplement of (*a*) dhokla made of wheat and Bengal gram providing mainly protein and energy and not much of vitamin A or calcium, (*b*) dhokla with leafy vegetables incorporated and (*c*) dhokla with both lime and leafy vegetables.

EXPERIMENTAL

Subjects and dietary supplement

The subjects in these investigations were seventy-two children aged 2–6 years attending a rural play centre. However, twenty-nine of them had participated in previous feeding experiments and results are, therefore, reported only for the remaining forty-three subjects who had not done so. They were divided into four groups matched for age, height, weight and other measurements (Tables 1–3). Groups 1–3 were given for 6 months food supplements, based on dhokla, which varied in calcium and vitamin A content; group 4 consisted of controls who were not given the supplement.

Traditionally dhokla is a fermented food prepared from rice and black gram (*Phaseolus Mungo* L. var. *radiatus*). In the feeding experiment wheat and Bengal gram, which are cheaper, were used and dhokla was prepared by mixing coarsely ground wheat and Bengal gram in the ratio 4:1 with water and salt to form a thick batter which was allowed to ferment overnight at room temperature.

For group 1, the fermented batter was poured into a greased pie dish, steamed (open steaming), cooled, cut into pieces and seasoned with mustard seeds fried in oil and garnished with chopped coriander leaves (1–1·5 g/serving). For group 2, 30 g of fenugreek leaves (*Trigonella foenum-graecum* L.) were mixed with the fermented batter prepared from 75 g of the wheat and Bengal gram mixture. The processing was otherwise the same. For group 3, in addition to 30 g of fenugreek leaves, 300 mg of lime powder were mixed with the fermented batter.

Dhokla was provided *ad lib.* between 13.00 and 14.00 hours and individual records were kept of the amounts consumed. As the children's main meals at home were at 10.00 and 20.00 hours, this did not interfere with food intake at home.

Studies were made after 6 months of the effect of the supplements on nutritional status as judged by food intake, height, weight and biochemical and skeletal status at the beginning and end of treatment.

Dietary intake

The mothers were asked how much of the different foods the child ate at different times of the day. They were asked to show the approximate quantity, and the food

Table 1. *Initial heights and weights of groups of children given a supplement of dhokla (group* 1), *dhokla with fenugreek leaves (group* 2), *dhokla with fenugreek leaves + lime (group* 3), *or no supplement (group* 4)

(Mean values with their standard errors)

Group no.	No. of subjects	Age (years)	Height (m)	Weight (kg)
1	11	4·1 (2·5–6·0)	0·955 ± 0·0232	11·7 ± 0·62
2	13	4·2 (3·0–5·5)	0·948 ± 0·0175	11·9 ± 0·35
3	10	3·9 (3·0–5·5)	0·935 ± 0·0230	12·0 ± 0·59
4	9	3·8 (2·5–5·0)	0·940 ± 0·0295	12·7 ± 0·71

Table 2. *Daily dietary intake (g) at home of groups of children given a supplement of dhokla (group* 1), *dhokla with fenugreek leaves (group* 2), *dhokla with fenugreek leaves + lime (group* 3), *or no supplement (group* 4)

	Foods						
Group no.	Cereals	Pulses	Leafy vegetable	Other vegetable	Milk	Oil	Sugar
1: at the beginning	156	16	3	47	110	12	17
at the end	152	15	4	49	110	12	17
2: at the beginning	150	15	4	52	100	14	20
at the end	150	15	4	53	100	14	19
3: at the beginning	154	17	3	50	115	15	15
at the end	158	17	3	51	120	16	14
4: at the beginning	150	15	4	53	105	14	16
at the end	149	16	4	54	100	14	16

intake was calculated from this. As the diets were monotonous (dal, chapati, vegetable, rice, kadi, tea) and standard cooking practices were used, it was possible to measure intake without much difficulty.

Height and weight

Erect body length was taken with the subject's heels, buttocks and upper back in contact with an upright board having an inlaid millimetre scale and a sliding horizontal bar that rests on the vertex. Weight was measured without shoes on a Detecto weighing machine (Detecto Scales Inc., New York, USA). The accuracy of the machine was checked frequently by calibration with standard weights.

Biochemical status

Blood and urine were collected at about 07.00 hours in the fasting state. Blood was collected by the finger-prick method and analysed for haemoglobin by the method of Evelyn & Malloy (1938). For the serum estimations blood was collected in capillary tubes and the serum was separated. Protein was estimated by the biuret method of Reinhold (1953) modified to microscale. Albumin was calculated from albumin:

Table 3. *Daily nutrient intake at home of groups of children given a supplement of dhokla (group 1), dhokla with fenugreek leaves (group 2), dhokla with fenugreek leaves + lime (group 3), or no supplement (group 4)*

Group no.	Energy (kJ)	Protein (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Vitamin A (retinol equivalent) (μg): As carotene*	As vitamin A	Total
1: at the beginning	3809	23·7	330	680	12·2	0·78	0·40	85	33	118
at the end	3726	23·5	328	665	11·8	0·75	0·39	91	33	124
2: at the beginning	3642	22·7	305	675	11·3	0·74	0·36	92	30	122
at the end	3621	23·0	315	670	11·7	0·75	0·37	95	30	125
3: at the beginning	3747	23·0	340	710	11·6	0·74	0·36	86	34	120
at the end	3830	23·6	338	700	12·1	0·77	0·36	87	36	123
4: at the beginning	3767	22·3	305	655	11·5	0·70	0·35	90	32	122
at the end	3767	22·2	307	665	11·6	0·70	0·35	92	30	122

* 4 μg carotene is taken as equivalent to 1 μg retinol on the basis of previous animal and human studies in this department.

Table 4. *Ingredients and nutrient content of the supplement given to children in the form of dhokla (group 1), dhokla with fenugreek leaves (group 2), dhokla with fenugreek leaves + lime (group 3), or no supplement (group 4)*

Group no.	Amount of dhokla consumed (g)	Ingredients of the supplement: Wheat (g)	Bengal gram (g)	Fenugreek leaves (g)	Lime powder (mg)	Nutrient content of the supplement: Energy (kJ)	Protein (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Carotene (as retinol equivalent) (μg)	Thiamin (mg)	Riboflavin (mg)
1	137	42	10	—	—	774	6·7	23	150	3·2	8	0·22	0·09
2	205	51	13	28	—	1026	9·2	95	210	8·2	315	0·31	0·14
3	230	64	16	35	330	1340	11·8	225	280	10·2	389	0·37	0·17
4	None	—	—	—	—	—	—	—	—	—	—	—	—

globulin ratio, determined by agar-gel electrophoresis according to the method of Varley (1958). Vitamin A was estimated by the method of Neeld & Pearson (1963).

Urine was collected in brown bottles containing about 1 g oxalic acid, and the bottles were transported under ice. Creatinine was estimated by the alkaline picrate method and nitrogen by the micro-Kjeldahl method (Hawk, Oser & Summerson, 1954). Vitamin C was estimated by the method of Roe & Kuether (1943), thiamin by the thiochrome method and riboflavin by the fluorimetric method (Association of Vitamin Chemists, 1951).

Skeletal status

Antero-posterior radiographs of hand and pelvis were taken before the beginning and at the end of the investigations. Bone age was judged by the method of Todd (1937). Cortical thickness of the second metacarpal was measured by the method of Garn (1966). Cortical thickness of the femur was measured at the distance of half the maximum pelvic width from the lowest point of contact between the acetabulum and the head of the femur.

RESULTS AND DISCUSSION

There was no significant change in dietary intake at home during treatment, as can be seen from the values in Tables 2 and 3. This might have been because the supplement was given 5 h after the morning meal and some 6 h before the evening meal. The intake of the supplement (dhokla) by the different groups at the play centre is shown in Table 4. As dhokla is a palatable moist food, the children were able to take appreciable amounts of it. Dhokla was chosen as a supplement partly because of the fact that in previous studies in this laboratory food intake was greater when dhokla was given than when other preparations, such as chapati and debra, containing the same ingredients in the same proportions were given (Sail, 1970).

The fermentation of dhokla batter is found to bring about increases in free sugar, amino nitrogen, water-soluble vitamins (thiamin, riboflavin, nicotinic acid and vitamin C) and ionizable iron, and a decrease in phytate phosphorus, suggesting an increase in digestibility. This might also contribute to the greater intake of dhokla.

The addition of lime and leafy vegetables was found to increase food intake and thereby nutrient intake. Dhokla incorporated with lime and leafy vegetables raised the intakes of energy, protein, calcium, iron and vitamin A to a satisfactory level. This supplement provided roughly one-quarter of the total energy intake (at home and at the play centre), one-third of the protein intake, two-thirds of the calcium intake, one-half of the iron intake and four-fifths of the carotene intake. The other varieties of dhokla provided somewhat less of these nutrients because of differences in food intake and the composition of dhokla.

An increase in food intake when diets deficient in calcium are supplemented with calcium has been found in our studies on rats as well as on schoolchildren. The increased weight gains observed at the Central Food Technological Research Institute, Mysore, in schoolchildren with administration of calcium-fortified salt (G. C. Jain, personal communication) must also be attributed to increased food intakes.

Table 5. *Changes in height and weight, biochemical status, composition of urine and skeletal status of groups of children given for a period of six months a supplement of dhokla (group 1), dhokla with fenugreek leaves (group 2), dhokla with fenugreek leaves + lime (group 3), or no supplement (group 4)*

(Mean values with their standard errors: nos of subjects in parentheses)

									Serum					
			Height (m)		Weight (kg)		Blood haemoglobin (g/l)		Protein (g/l)		Albumin (g/l)		Vitamin A (retinol equivalent) (μg/l)	
Group no.	Measurement	Age (years)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1 (11)	Initial	4·1	0·955	0·0232	11·7	0·62	92	2·4	58	2·0	31	1·3	230	17
	Increment	0·5	0·030*	0·0024	1·1**	0·09	3	0·8	1	0·6	1	0·5	10	10
2 (13)	Initial	4·2	0·948	0·0176	11·9	0·35	90	2·8	56	1·5	29	0·8	190	13
	Increment	0·5	0·031**	0·0011	1·2**	0·12	6*	1·1	3**	0·5	3**	0·3	70**	3
3 (10)	Initial	3·9	0·955	0·0230	12·0	0·59	92	2·9	60	1·6	31	1·0	230	26
	Increment	0·5	0·032**	0·0030	1·5**	0·08	8**	1·3	2*	0·6	3*	0·7	90**	9
4 (9)	Initial	3·8	0·940	0·0295	12·7	0·71	96	3·5	59	1·4	32	1·7	260	7
	Increment	0·5	0·020	0·0017	0·2	0·01	0	1·4	0	0·3	0	0·5	−20	13

Table 5 (*cont.*)

Group no.	Measurement	Urine: Creatinine (mg/l) Mean	SE	Vitamin C (mg)* Mean	SE	Thiamin (μg)* Mean	SE	Riboflavin (μg)* Mean	SE	Nitrogen (mg)* Mean	SE
1 (11)	Initial	335	29·5	0·0352	0·0055	1·014	0·147	0·666	0·041	7·6	1·1
	Increment	11	6·9	0·0008	0·0014	0·081	0·038	0·114	0·024	0·6	0·3
2 (13)	Initial	298	19·2	0·0370	0·0052	0·973	0·079	0·665	0·054	8·9	0·4
	Increment	13*	4·4	0·0004	0·0023	0·070	0·033	0·125	0·031	0·0	0·1
3 (10)	Initial	282	12·7	0·0332	0·0067	1·180	0·135	0·884	0·062	10·8	1·0
	Increment	25*	7·4	0·0058*	0·0011	0·036	0·057	0·147	0·026	0·2	0·2
4 (9)	Initial	308	15·8	0·0232	0·0061	1·034	0·128	0·699	0·059	9·2	1·1
	Increment	−6	5·0	0·0006	0·0027	−0·008	0·045	0·057	0·037	0·1	0·2

(Values/mg of creatinine)

Group no.	Measurement	Skeleton: Bone age (years) Mean	SE	Bone age × 100 / Chronological age Mean	SE	Cortical thickness (mm): 2nd metacarpal bone Mean	SE	Cortical thickness (mm): Femur Mean	SE
1 (11)	Initial	3·0	0·41	71	6·2	1·6	0·09	4·9	0·45
	Increment	0·4	0·12	4*	2·8	0·2*	0·04	0·5*	0·10
2 (13)	Initial	2·8	0·25	67	3·7	1·7	0·06	4·4	0·21
	Increment	0·6*	0·11	5*	2·0	0·4**	0·03	0·8**	0·07
3 (10)	Initial	2·8	0·27	72	5·2	1·5	0·06	4·6	0·34
	Increment	0·9**	0·09	12**	1·9	0·6**	0·03	1·0**	0·11
4 (9)	Initial	3·1	0·45	82	7·1	1·6	0·16	4·1	0·19
	Increment	0·3	0·06	−5	2·3	0·1	0·02	0·2	0·03

* Significantly greater than control value ($P < 0·05$).
** Significantly greater than control value ($P < 0·001$).

Venkatachalam (1971), however, observed no increase in weight gain in other studies with calcium supplementation; this may have been due to differences in the phosphorus content of the diet and the vitamin D status of the subjects. It is also possible that the improvement in taste brought about by incorporation of lime was responsible for the increased intake. Sensory trials showed that dhokla with incorporated lime was more acceptable, probably because of the reduced acidity.

Height and weight

The children were about 0·94 m tall and weighed about 12 kg at the beginning of the experiment, compared with the expected value in upper-class children of about 0·98 m and 14 kg, on the basis of norms previously obtained in this laboratory. All the groups given the supplement gained more weight (Table 5) than the controls, but group 3, who were given dhokla enriched with both lime and greens, gained the most weight. This may have been partly or wholly due to differences in food intake.

The weight gain of 0·2 kg in the control group is less than the value of about 0·5 kg obtained in other studies. Two subjects in this group lost about 0·5 kg, two maintained stationary weights and the others gained about 0·5 kg.

Haemoglobin

As in previous studies, the response of blood haemoglobin concentration to supplementation was not large, but was greatest in group 3 and least in group 1 (Table 5). However, when the values were classified according to the norms of the Interdepartmental Committee on Nutrition for National Defense (1963) the number of deficient values declined from 91 to 82% in group 1, from 92 to 69% in group 2 and from 88 to 25% in group 3. None of the values in any of the three experimental groups were in the acceptable range either before or after the treatment. In the control group the number of deficient values declined from 71 to 57% but the number of acceptable values also declined from 29 to 0%, and there was no general improvement. The slow response of blood haemoglobin concentrations to nutritional improvement has been noticed in other studies in this laboratory (Rajalakshmi and associates, unpublished) as well as by other investigators (Dumm, Rao, Jesudian & Benjamin, 1966*a*, *b*; Devadas, Anandam & Bhanumathi, 1967; Devadas, Espen & Susheela, 1968).

Serum protein, albumin and vitamin A

The different groups did not vary appreciably with regard to mean values for serum total protein (Table 5). None of the values was in the high range either before or after treatment. The number of acceptable values increased from 9 to 18% in group 1, from 15 to 31% in group 2 and from 25 to 57% in group 3. There was a corresponding decline in the number of either deficient or low values or both. The number of acceptable values did not increase in the control group.

Serum albumin and vitamin A values followed essentially the same pattern (Table 5). Again, no values for serum albumin were found in the high range either before or after the treatment in any of the groups. The percentage of acceptable values increased in groups 2 and 3 from 0 and 12 to 31 and 63 respectively. The groups given leafy vegetables showed significant increments in serum vitamin A. However, none of the values were in the deficiency range, even initially, in any of the groups and the number of values in the low range declined in all the three experimental groups. Vitamin A deficiency was found to be less evident in preschool children than in children of school age in previous studies in this laboratory. This may be because the young children in this region get relatively more vitamin A and carotene in proportion to body-weight and energy intake than schoolchildren.

Urinary excretion of creatinine, nitrogen and vitamins

Here again the biggest increases were found in group 3 and the smallest increases in group 1, with the controls showing no change (Table 5). The better response of group 3 compared with group 2 may have been due to increased food intake as supplementation with calcium *per se* cannot account for the results.

Skeletal status

All the fed groups given the supplement showed greater increments in bone age as well as cortical thickness of the second metacarpal bone and femur than the controls (Table 5). The size of the increments increased from group 1 to group 3, as might be expected.

The percentages of children showing a bone age increment of more than 1 year were 9 in group 1, 23 in group 2, 50 in group 3 and none in group 4.

A similar pattern was observed for cortical thickness of second metacarpal bone, with 60% of the children in group 3 showing an increase of more than 0·5 mm as against 8% in group 2 and none in groups 1 and 4. A similar pattern was also found for cortical thickness of the femur.

These studies suggest that the skeletal retardation found in young children is only partly due to protein-energy deficiency and that calcium deficiency is a crucial factor. Children in group 3 receiving a total of 580 mg Ca and 980 mg phosphorus and no vitamin D showed a good improvement in skeletal status, suggesting that, at these levels of calcium and phosphorus in diets which provide reasonably satisfactory amounts of energy and protein, vitamin D is not crucial, although more recent studies suggest that when the diet is limiting in either calcium or phosphorus, and exposure to sunlight is more restricted, supplementation with vitamin D may help in this age group (Rajalakshmi and associates, unpublished findings).

In conclusion, these studies demonstrate the feasibility of improving the nutritional status of young children by a simple supplement based on locally available plant foods, providing adequate daily amounts of energy (1340 kJ), protein (12 g), calcium (225 mg), vitamin A (389 μg) and other nutrients. They also suggest that calcium deficiency is a crucial factor in the aetiology of skeletal retardation in young children.

This work was done under Am PL 480 project FG-In-402 sponsored by the USDA, Washington. We thank Dr N. A. Dave of the Radiology Department, S. S. G. Hospital, Baroda for his help in radiological examination.

REFERENCES

Association of Vitamin Chemists (1951). *Methods of Vitamin Assay* 2nd ed. New York: Interscience Publishers.

Devadas, R. P., Anandam, K. & Bhanumathi, L. (1967). *J. Nutr. Diet., India* **4**, 51.

Devadas, R. P., Espen, M. & Susheela, A. (1968). *J. Nutr. Diet., India* **5**, 206.

Dumm, M. E., Rao, B. R. H., Jesudian, G. & Benjamin, V. (1966*a*). *J. Nutr. Diet., India* **3**, 25.

Dumm, M. E., Rao, B. R. H., Jesudian, G. & Benjamin, V. (1966*b*). *J. Nutr. Diet., India* **3**, 111.

Evelyn, K. A. & Malloy, H. T. (1938). *J. biol. Chem.* **126**, 655.

Garn, S. M. (1966). *Publs natn. Res. Coun., Wash.* no. 1282, p. 43.

Hawk, P. B., Oser, B. L. & Summerson, W. H. (1954). *Practical Physiological Chemistry* 13th ed. New York: The Blakiston Company.

Inter-departmental Committee on Nutrition for National Defense (1963). *A Manual of Nutrition surveys.* Washington, DC: USA Government Printing Office.

Neeld, J. B. Jr & Pearson, W. N. (1963). *J. Nutr.* **79**, 454.

Rajalakshmi, R. & Ramakrishnan, C. V. (1967). *Int. Congr. Nutr.* VII *Hamburg* Vol. 3, p. 95.

Ramachandran, K. (1968). Formulation and evaluation of low cost balanced meals based on locally available foods for children in the post-weaning period. Thesis submitted for PhD (Biochemistry) degree, M.S. University, Baroda.

Reinhold, J. G. (1953). In *Standard Methods of Clinical Chemistry* Vol. 1, p. 88. Quoted by Varley, H. (1958) *Practical Clinical Biochemistry*. London: William Heinmann Medical Books Ltd.

Roe, J. H. & Kuether, C. A. (1943). *J. biol. Chem.* **147**, 399.

Sail, S. S. (1970). Nutritional studies on school children. Thesis submitted for PhD (Foods and Nutrition) degree, M.S. University, Baroda.

Todd, T. W. (1937). *Atlas of Skeletal Maturation*. Quoted in *Roentgen Signs in Clinical Practice* Vol. 1 [I. Meschan and R. F. Meschan, editors]. Philadelphia: W. R. Saunders Company.

Varley, H. (1958). *Practical Clinical Biochemistry*. London: William Heinmann Medical Books Ltd.

Venkatachalam, P. S. (1971). *Proc. Nutr. Soc. India* **10**, 22.

Printed in Great Britain