# Effect of supplementation of micronutrient fortified biscuits on serum total proteins and vitamin A levels of adolescent girls (10-16 years) of Jaipur city, India

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## ABSTRACT

Vitamin A deficiency is widely prevalent amongst women and children in India. The aim of the study is to study the effect of supplementation of micronutrient fortified biscuits on serum total proteins and vitamin A levels of adolescent girls (n=46, 10-16 years) studying in a government school in Jaipur city, India. The study was designed to be an intervention study. The intervention was with biscuits containing 11.4 g of protein and fortified with 600 mcg vitamin A, 30 mg iron, 100 mcg folic acid, 40 mg vitamin C and 150 mcg iodine per day for all working days in a 4 month period. The results indicated that the protein status of 93.5% of the adolescent girls was adequate prior to intervention, this percentage increased to 97.8% at post intervention. Vitamin A supplementation augmented the percentage of adolescent girls in the 'normal' category from 56.1% to 73.2% and decreased the percentage of adolescent girls in the 'low' category from 41.5% to 26.8%. One girl who was in the 'deficient' category moved to the 'low' category. Hence, supplementation with biscuits fortified with vitamin A and other micronutrients improved the vitamin A status of the adolescent girls markedly. It is, therefore, recommended that the school system can be used for micronutrient supplementation to improve the micronutrient status of children and adolescents as the students are more regimented here for distribution of nutrient fortified food products.

**Keywords:** Serum total proteins, serum vitamin A, adolescent girls, Jaipur city.

## INTRODUCTION

Micronutrient deficiency is a serious health concern in most developing countries. In India, iron deficiency, vitamin A deficiency and iodine deficiency disorders are of greatest public health significance. Vitamin A deficiency has been recognized to be a major controllable public health and nutritional problem. An estimated 5.7% children in India suffer from eye signs of vitamin A deficiency. Even mild vitamin A deficiency probably increases morbidity and mortality in children, emphasizing the public health importance of this disorder.[1] In a recent survey, the overall prevalence of bitot spots was found to be about 0.8% in <5 year old children (n=71,591) from eight states viz., Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Orissa, Tamil Nadu, Maharashtra and West Bengal in the years 2002-03 and about 61.8% of 1-5 year old children (n=3,934) had vitamin A levels <20 mcg/dl.[2]

Supplementation with vitamin A, iron and iodine alone or in combination has shown beneficial effects on the nutritional status of children and adolescents. Vitamin A/vitamin A food based supplementation has improved serum vitamin A/retinol levels especially in vitamin A deficient children.[3,4] High dose of vitamin A supplementation (206,000 IU) every four months for 1 year had improved linear growth of children with very low serum retinol levels although the effect was modified by age and breast feeding.[5] In another study, supplementation with 60 mg of oily solution of retinyl palmitate every 6 months for 1 year increased growth of malnourished children in eastern Zaire[6] and so did supplementation with 60,000 retinol equivalents in vitamin A deficient children in Nepal.[7] Vitamin A supplementation with iron/and folic acid[8,9] and other micronutrients[10] had improved growth and iron status in young children and adolescents.

Multiple micronutrient supplementation has been used for improving anaemia, micronutrient status, growth and morbidity in children and adolescents. van Stuijvenberg and Benade[11] had used a biscuit which was fortified with beta carotene, iodine and iron with a cold drink as a carrier for vitamin C to address micronutrient deficiencies in primary school children in South Africa. The 12 month intervention resulted in a significant improvement in blood levels of vitamin A, ferritin, iron, haemoglobin, haematocrit and in urinary iodine levels.[12,13] Multiple micronutrient supplementation for improving micronutrient status has also been used in Peru and South Africa.[14,15]

In India, Gopaldas[16,17] had improved the mid-day meal/ school lunch programme in Gujarat with the addition of a 'package' of health inputs, including anthelminthics and micronutrient supplementation of iron and vitamin A, and iodine fortified salt in 1993. The children as a result had better growth and micronutrient status. Gopaldas and Gujral[18,19] had empowered a tea plantation community in South Africa to improve its micronutrient health by taking iron and vitamin A tablets and using iodized salt.

The intake of nutrients like vitamin A, iron and iodine can be increased by fortifying snack items well liked by children. van Stuijvenberg[20] has advocated that school feeding can be used as a vehicle for micronutrient fortification. Snack items can be introduced in schools catering to the deprived section of our society. A biscuit is seen as a snack and is easy to distribute and has long shelf life. It is also easy to monitor and therefore, less open to misuse and corruption.[11]

Multiple micronutrient malnutrition is most rampant in slums. Adolescent girls are a marginalized group in any society more so when they belong to the low socio economic group and come from slums. The present study was designed to be an intervention study where biscuits containing 11.4 g of protein and fortified with nutrients as vitamin A, iron, folic acid, iodine and ascorbic acid were used to supplement the diet of adolescent girls attending a government school. The girls suffer from malnutrition and have multiple micronutrient deficiencies, hence, this study was an attempt to ameliorate their nutritional status.

## MATERIALS AND METHODS

Ten government schools in Jaipur city were visited. The willingness of the Principal of the school to participate in the study, adequate number of girl students in a separate section in higher classes and the school being close to the University campus for logistic reasons were the points for consideration in the selection of the school. All adolescent girls (n=148, 10-16 years) studying in classes VI to VIII attending a government school fulfilling all criteria, and residing in a slum where the school was situated comprised the sample for the study. However, pre and post data of 46 adolescent girls (10-16 years) for serum total proteins and vitamin A were available for analysis.

Biscuits were prepared with 35 g wheat flour whole, 15 g soyabean flour, 30 g sugar, 20 g of hydrogenated fat and 20 ml of milk. The biscuits furnished 497 kcal and 11.4 g of protein. Hundred grams of biscuits fortified with vitamin A (600 mcg), iron (30 mg), folic acid (100 mcg), vitamin C (40 mg) and iodine (150 mcg) at one RDA levels were provided to adolescent girls (n=46). The biscuits were sent to Food Research and Analysis Centre, Federation House, Tansen Marg, New Delhi for determining the losses of nutrients on baking. The biscuits were prepared with added nutrients taking losses into consideration.

**Table-1:** Effect of supplementation on serum total protein levels of adolescent girls

| | Pre intervention | | | Post intervention | | | |
|---|---|---|---|---|---|---|---|
| | Total Protein (g%) | Normal (>=6.0g%) | Low (<6.0g%) | Total Protein (g%) | Normal (>=6.0g%) | Low (<6.0g%) | Paired t test value |
| Total Sample (n=46) | 6.8 ±0.571 | 43 (93.5) | 3 (6.5) | 6.89 ±0.417 | 45 (97.8) | 1 (2.2) | 1.544 NS |
| 10+ -11+ years (n=11) | 6.3 ±0.243 | 9 (81.8) | 2 (18.2) | 6.91 ±0.342 | 11 (100.0) | 0 | 4.975* |
| 12+ -13+ years (n=23) | 6.7 ±0.534 | 22 (95.7) | 1 (4.3) | 6.77 ±0.398 | 22 (95.7) | 1 (4.3) | 0.403NS |
| 14+ -16+ years (n=12) | 7.2 ±0.535 | 12 (100.0) | 0 | 7.11 ±0.450 | 12 (100.0) | 0 | 0.538 NS |

Mean ± SD, Figures in parentheses denote percentages, NS Non Significant, *Significant at 5% level of significance

Biscuit distribution commenced from 1st September 2004 and continued till 18th December 2004 for all working days. Considering the holidays during this period, the biscuits were distributed for a total of 75 days in these four months. The mean attendance of the girls was 74.3±17.015 percent. The school had closed for winter vacations from 23rd December 2004, hence blood collection was made on the 22nd and 23rd December 2004. Pre intervention data were collected in the month of August 2004. Prior to intervention the adolescent girls were dewormed with 400mg of albendazole. The subjects were asked to take the tablets in the school in front of the investigator.

Blood collection was carried out in Jaipur and the blood samples were analysed for serum total proteins and vitamin A at All India Institute of Medical Sciences, New Delhi. Blood was collected in labeled plain vials. Serum was separated from blood in plain vials and transferred to labeled vials for transport to New Delhi for analysis packed with dry ice in thermocole boxes. Total protein was estimated by the biuret method as per the kit of Randox Laboratories Ltd., UK. Serum vitamin A was analysed by an improved trifluoroacetic acid method as proposed by Bradley and Hornbeck.[21] The data were collected from July 2004 to December 2004.

Acceptable levels of serum proteins for 6-17 year old children were considered to be ≥ 6.0 g/100 ml, and low levels when these were below 6.0g/100 ml.[22] The cut off points for serum retinol were taken as < 10 mcg/dl as deficient and between 10-19 mcg/dl as low. The acceptable values were taken to be between 20-49 mcg/dl.[23] Low serum vitamin A values are suggestive of a depleted state, with or without the presence of clinical signs of deficiency.

The study was approved by the Departmental Ethics Committee and a written consent was obtained from the parents of the students for participation in the study.

## RESULTS

**Effect of supplementation on serum protein levels of adolescent girls:** The mean serum protein levels of the adolescent girls at pre and post intervention were in the normal category with serum protein levels greater than 6.0g% (Table-1). No agewise variation was observed in mean serum total protein levels of the subjects, the mean serum total protein levels ranged between 6.3±0.243g% to 7.2±0.535g%. The serum protein status of the adolescent girls was found to be adequate.

On intervention with biscuits it was observed that 2 adolescent girls who were in the 'low' category had moved to the 'normal' category, thereby, taking the percentage of adolescent girls from 93.5% at pre intervention to 97.8% at post intervention in the 'normal' category. Hence, intervention had made slight improvement in the protein status.

**Effect of supplementation on serum vitamin A levels of adolescent girls:** The mean serum vitamin A levels of the adolescent girls had increased from 20.6±6.385 mg/dl to 25.5±7.083 mg/dl after intervention. Adolescent girls in the higher age groups had lower vitamin A levels, however, increases were observed after supplementation with 600 mcg of vitamin A per day for about 4 months.

Vitamin A supplementation resulted in increasing the percentage from 56.1% to 73.2% in the 'normal' category at post intervention and decreasing the percentage of subjects in the 'low' category from 41.5% to 26.8%

**Table-2:** Effect of supplementation on serum vitamin A levels of adolescent girls

| | Pre intervention | | | | Post intervention | | | |
|---|---|---|---|---|---|---|---|---|
| | Serum vitamin A (mcg/dl) | Normal (>=20mcg/dl) | Low (10-19mcg/dl) | Deficient (<10mcg/dl) | Serum vitamin A (mcg/dl) | Normal (>=20mcg/dl) | Low (10-19mcg/dl) | Paired t test value |
| Total Sample (n=41) | 20.6 ±6.385 | 23 (56.1) | 17 (41.5) | 1 (2.4) | 25.5 ±7.083 | 30 (73.2) | 11 (26.8) | 4.110* |
| 10+ -11+ years (n=10) | 25.4 ±7.341 | 8 (80.0) | 2 (20.0) | 0 | 28.2 ±9.761 | 8 (80.0) | 2 (20.0) | 0.988 NS |
| 12+ -13+ years (n=19) | 19.7 ±4.101 | 12 (63.2) | 7 (36.8) | 0 | 24.3 ±6.427 | 14 (73.7) | 5 (26.3) | 3.079* |
| 14+ -16+ years (n=12) | 18.1 ±6.880 | 2 (16.7) | 9 (75.0) | 1 (8.3) | 25.3 ±5.245 | 10 (83.3) | 2 (16.7) | 3.031* |

Mean ± SD, Figures in parentheses denote percentages, NS Non Significant, *Significant at 5% level of significance

(Table-2). Moreover, one 'deficient' subject after ameliorating her vitamin A status moved to the 'low' category. However, intervention could not make all the subjects vitamin A sufficient. It is, therefore, suggested that either the dose or the period of intervention be enhanced.

## DISCUSSION

Jood *et al*[4] studied the effect of supplementation on serum retinol levels in 66 school children 10-12 years of age from Hisar, India, for a period of about 4 months. Hundred grams of cauliflower leaves powder supplements ie., biscuits and shakarparas were fed to 33 deficient subjects (Hb <10 g/dl and serum retinol <20 mcg/dl). An increase of 33.27% was observed in the serum retinol levels. Hence, intervention with cauliflower leaves powder was found to be effective in raising serum retinol levels over a period of about 4 months. In the present study, supplementation with 600 mcg vitamin A for about 4 months had resulted in an increase of 23.92% in the mean serum vitamin A levels of adolescent girls.

Kumar and Rajagopalan[24] evaluated the impact of a multi-micronutrient food supplement on children (n=82, 5-15 years) from Chennai, India. At the end of 9 months of intervention, significant improvements in the mean serum vitamin A levels from 47.18± 19.66 to 58.33± 25.82 mcg/dl were observed. Varma *et al*[25] assessed the efficacy of a premix fortified with iron and vitamin A added to prepared khichadi (a rice and pulse preparation) for 24 weeks on children (n=516, 36-66 months) from West Bengal, India. After supplementation for 24 weeks serum retinol had significantly increased from 1.1±0.4 mcg/l to 1.4±0.4 mcg/l. Moreover, vitamin A deficiency defined as serum retinol <0.70 mcg/l had reduced from 17.5% to 8.1% and the low vitamin A status defined as 0.70 > serum retinol <1.05 mcg/l had declined from 47.9% to 21.5%. Such improvements were also observed in the present study.

Gopaldas[17] reported that the mid-day meal or school lunch programme in Gujarat, India, was improved from 1994 with the addition of a package of health inputs, including anthelminthics and micronutrient supplementation of iron and vitamin A, and iodine fortified salt. The evaluation of the improved mid day programme revealed that the prevalence of night blindness and vitamin A deficiency had reduced from 67% to 34%. Women tea pickers and their families were empowered to take iron (60 mg of elemental iron two times a week) and vitamin A (1,600 IU, once a week), and to purchase subsidized iodized salt (30 ppm) from the plantation ration shop in South India.[18] At the end of nine months of intervention the prevalence of clinical signs of vitamin A deficiency in the entire plantation population (about 2,500) had reduced significantly from 19% to 4% and common health problems had decreased from 88% to 54%. Benefits of intervention with vitamin A were also demonstrated in the present study, too.

Ahmed *et al*[9] conducted a study to determine whether concomitant supplemental vitamin A enhanced the response to weekly supplemented iron and folic acid in anaemic teenagers in urban Bangladesh. Participants with Hb concentration of 8-12g/dl received supplements of placebo, vitamin A, or iron + folic acid + vitamin A weekly for 12 weeks. The supplements contained 2.42 mg vitamin A (retinol) as retinyl palmitate, 120 mg elemental iron as ferrous sulphate, and 3.5 mg folic acid. Vitamin A supplementation increased the serum vitamin levels from 0.89± 0.27 micromol/l to 1.04±0.27 micromol/l and iron + folic acid + vitamin A increased the vitamin A levels from 0.96±0.29 micromol/l to 1.09± 0.24 micromol/l. Vitamin A supplementation alone reduced vitamin A deficiency from 73% to 54% and iron + folic acid + vitamin A decreased vitamin A deficiency from 66% to 45%. Hence, vitamin A alone or with iron and folic acid was effective in improving the vitamin A status of teenage girls. In the present study, too, vitamin A supplementation at one RDA level improved the vitamin A status of the adolescent girls. The percentage of girls in the 'normal' category had increased from 56.1% to 73.2% on supplementation. However, vitamin A was supplemented together with iron, folic acid, vitamin C and iodine.

van Stuijvenberg *et al*[12] determined the effect of micronutrient fortified biscuit on the micro nutrient status of primary school children from a rural community in South Africa. Micronutrient status was assessed in 115 children aged 6-11 years before and after consumption of biscuit (fortified with iron, iodine and beta carotene) for 43 weeks over a 12 month period. The serum retinol levels had increased significantly from the baseline levels of 0.74 mmol/l to 0.87 mmol/l after intervention. Moreover, the prevalence of children with low serum retinol concentration <0.70 mmol/l) had decreased from 39.1% to 12.2% after 12 months of intervention. In the present study, too, the mean serum retinol levels had increased significantly from 20.61±6.385 mcg/dl to 25.54±7.083 mcg/dl and the adolescent girls in the 'low' category had decreased from 41.5% to 26.8%.

In Tanzania, in a well conducted randomised double blind placebo controlled trial, a dietary supplement in the form of a fortified powder fruit drink was evaluated in school children. Children consumed for six months 25 g/school day attended, the powder being added to 200 ml of water. The dietary supplement provided between 40 and 100% of the RDA for 10 micronutrients, which included iron, vitamin A and iodine. The dietary supplement produced statistically significant differences not only in vitamin A and iron status, but also in the growth of young school age children.[10] Significant improvements in vitamin A status of adolescent girls was also recorded in the present study. However, as has been reported earlier [26,27],it is worth while also to consider the intestinal parasitic infection and do periodic desorbing specially in the community with poor hygienic and sanitary conditions as simply deworming resulted in significant increases in the retinol, B carotene and hemoglobin levels.

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