**Effects of a food-based intervention on markers of micronutrient status among Indian women of low socio-economic status**

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**ABSTRACT (246 words)**

Intakes of micronutrient-rich foods are low among Indian women of reproductive age. We investigated whether consumption of a food-based micronutrient-rich snack increased markers of blood micronutrient concentrations when compared with a control snack. Non-pregnant women (n=222) aged 14-35 years living in a Mumbai slum were randomised to receive a treatment snack (containing green leafy vegetables, fruit and milk powder) or a control snack containing foods of low micronutrient content such as potato and tapioca. The snacks were consumed 6 days/ week for 12 weeks, and blood was collected at 0 and 12 weeks. Food frequency data were collected at both time points. We assessed the effect of group allocation on change in blood nutrient concentration using univariate and multivariate regression models. Compliance (defined as proportion of women who consumed ≥3 snacks/week) was >85% in both groups. Median (IQR) β-carotene concentrations in the treatment group increased from 385 (323, 470) nmol/L at 0 weeks to 470 (380, 610) nmol/L at 12 weeks. The median difference between control and treatment groups in the change in concentrations was 48.7 (7.4, 89.7) nmol/L. Allocation to the treatment group was a positive independent predictor of change in β-carotene concentrations (p=0.023). There was no effect of group allocation on concentrations of ferritin, retinol, ascorbate or folate. This study shows that locally sourced foods can be made into acceptable snacks that increase the β-carotene status of women of reproductive age. However there was no increase in circulating concentrations of the other nutrients measured.

**INTRODUCTION**

Multiple micronutrient deficiencies are prevalent among women of reproductive age in India (1-3). There are limited data on the micronutrient status of women living in Indian slums. However, there is evidence that such women are at risk of deficiency. A recent review (4) of studies investigating the intake of micronutrients among women of child bearing age in low income countries found that in Asia, intakes of several micronutrients were frequently below the Estimated Average Requirement (EAR). The majority of studies reviewed found that intakes of iron and folate were less than half of the EAR and analysis of dietary intake data showed that between 25-77% of women in Asia had average intakes of vitamin A and vitamin C below the EAR. No data on carotenoid intakes were reported in the review. The authors suggested that these findings may reflect the cereal-based diets low in fruit, vegetable and animal foods that are commonly consumed in such populations.

 Animal foods are expensive (5) and a large proportion of the Indian population are vegetarian. Milk, fruit and green leafy vegetables are a good source of micronutrients but intakes of these foods are low, with national survey data indicating that in 2005-6 over 50% of Indian women consumed fruit less than once per week (6). When these data were broken down by region and demographic group, over two thirds of women living in Mumbai slum areas (n= 803) consumed green leafy vegetables on a daily basis. However, only frequency data were collected in the survey and several Indian preparations contain very small amounts of green leafy vegetables (<5g) such as coriander for seasoning. It is therefore likely that the proportion of women who consumed a 100g portion daily as recommended in the Indian National Institute of Nutrition guidelines (7) would be considerably smaller than this. A quarter of women reported consuming fruit daily and approximately 40% ate fruit less than once a week. Over 15% of women never consumed milk or curd (yoghurt) and 56% consumed these items less than once a week. Recently, quantitative data on dietary intake has been collected from women living in North Indian slums which shows that intakes of fruit and vegetables were very low (8; 9). Anand et al found that in slum areas of Haryana state, the mean number of servings per day of fruit and vegetables was 2.2 for women, with only 5.4% of women consuming 5 servings per day (9).

 We aimed to assess the effect of daily consumption of a food-based intervention in the form of a snack food containing green leafy vegetables, fruit and milk on blood micronutrient concentrations in non-pregnant low income Indian women of reproductive age.

**METHODS**

The present study was conducted between October 2009 and March 2010 and was an adjunct to a larger on-going randomised controlled trial; the Mumbai Maternal Nutrition Project (MMNP)(10) which was launched in January 2006 (Trial registration: ISRCTN 62811278). The MMNP intervention was based on the results of the Pune Maternal Nutrition Study (PMNS), an observational study in a rural area near the city of Pune, which reported that maternal intakes of green leafy vegetables, fruit and milk during pregnancy were positively associated with offspring birth size measurements (11). The MMNP was designed to investigate the effect of consumption of a snack containing green leafy vegetables, fruit and milk for at least 3 months prior to conception and throughout pregnancy on infant size and mortality. The treatment snack was designed such that in addition to the women’s habitual diet, a daily intake of ≥75th centile of green leafy vegetable, fruit and milk reported in the PMNS would be achieved.

 The present study was a randomised controlled trial of the MMNP intervention and was undertaken to examine its effect on micronutrient status among the women. This was not assessed in the main MMNP trial to minimize blood taking which was thought likely to deter women from taking part. The present study was conducted approximately 10 miles from the MMNP study area and the participants were not enrolled in the MMNP at any time.

Ethical approval for the present study was obtained from JJ Hospital Ethics Committee, Mumbai.

*Participants and Setting.* The participants lived in the Shivaji Nagar slum area of Mumbai with inadequate access to safe water and sanitation, poor structural quality of housing and overcrowding. These criteria have been used by the United Nations to define slums (12). Women were not eligible for the study if they were <14 or >35 years of age, or reported that they were pregnant or breast-feeding at the time of enrolment. There is a significant migration rate in the study area so we asked women to enrol only if they intended to remain in the study area for at least 3 months. A power calculation showed that a sample size of 82 per group was necessary to demonstrate a difference in the proportion of vitamin A deficiency (defined as serum retinol <0.200ng/ml) of the order of 35% in the control group versus 10% in the treatment group at 90% power and at the p=0.05 significance level (13). These figures were based on data from the World Health Organisation Vitamin and Mineral Information System database whereby the prevalence of vitamin A deficiency ranged from 33-35% in a study of 150 women in urban Calcutta (14). Assuming a drop-out rate of 20%, our target sample was 102 in each group.

*Procedure***.** In October 2009, women living in the Shivaji Nagar area were invited to community meetings at which they were informed about the study by the research team. Informed written consent was obtained from all women who agreed to participate. Those who met the inclusion criteria (n=222) were enrolled and randomised stratified by age and BMI to one of two treatment or two control groups. The women were given colour coded ID cards based on group allocation which were used to ensure they received the correct snack. Baseline data and venous blood samples were collected prior to the start of supplementation and within 7 days of enrolment. Demographic data and information on synthetic micronutrient supplement intake in the form of tablets or tonics were collected by questionnaire. A 221-item interviewer-administered food frequency questionnaire (FFQ) developed for this population (10) was used to collect data on the past week’s green leafy vegetable and fruit intake at baseline and 12 weeks. This was in order to assess any changes in intakes of these foods over the supplementation period. Height was measured to the nearest mm using a portable stadiometer (Microtoise, UK) and weight to the nearest 100 g using electronic scales (Salter, UK).

 During the 12 week supplementation period women were asked to come to a local community centre six days per week (Monday – Saturday) to receive the snacks and consume them under observation. The centres were ≤10 minutes walk from the women’s homes. Consumption of the snacks was recorded by a project health worker. Compliance with the intervention was defined as consumption of a mean of ≥3 supplements per week over the 12 week period. Further blood samples were collected 12 weeks after supplementation started. Any women found to be moderately or severely anaemic (Hb<10g/dL) at baseline or 12 weeks were offered iron tablets. Women who were mildly anaemic (Hb = 10-11.99g/dL) were informed of their status and advised to see a doctor and consider taking iron supplements. Anaemic women were eligible to remain in the study.

*Intervention.* The intervention was one cooked snack per day such as a ‘samosa’ or ‘patty’ made from locally available food ingredients. The treatment snacks contained approximately 25g fresh green leafy vegetables (e.g. spinach, colocasia, coriander, fenugreek leaves), 10g dried fruit (e.g. figs, dates, raisins) and 12g whole milk powder. The control snacks contained foods such as potato, sago or tapioca. Both types of snack were prepared with binding ingredients such as wheat or corn flour, and spices were added. The snacks were cooked by shallow frying in sunflower oil. Several varieties of the treatment (n=5) and control snacks (n=7) were developed, to provide variety. The average weight of the snacks was approximately 65g (treatment) and 36g (control). The energy and micronutrient content of the snacks is shown in Table 1. The women were advised to consume the snacks in addition to their habitual diet. One snack per day was given to the women at a time least likely to interfere with their usual intake; between 3:00pm and 6:00pm, Monday to Saturday.

*Blinding.* The treatment and control snacks were outwardly similar but their contents looked different. To achieve a degree of allocation concealment, we created two treatment and two control groups, each with an independent set of recipes. Four different snacks were produced daily, in an unpredictable pattern. The snacks were packaged in colour coded bags to ensure that they were correctly received by the women. Staff who measured outcomes were blind to the women’s allocation group. The two treatment groups and two control groups were merged for analysis.

*Nutrient content of the intervention.* Prior to nutrient analysis, snacks were frozen in Mumbai and transported on dry ice to the UK where they were analysed at Eclipse Laboratories (Cambridge, UK) for micronutrient content (**Table 1**).

 To assess mineral content samples were dried and ashed at 550°C for 16 hours, then dissolved in 5M hydrochloric acid and scandium internal standard/ caesium chloride solution was added. After filtration and dilution to known volumes with water, the concentration of each mineral was determined by ‘Liberty series II’ Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

 Ascorbic acid was extracted from the sample using metaphosphoric acid and EDTA. The ascorbic acid was then enzymatically oxidised to dehydro ascorbic acid which was condensed with O-phenylene diamine to the fluorescent quinoxaline derivative. The latter was separated from interfering compounds by reverse phase HPLC with fluorimetric detection.

 Β-carotene analysis was conducted away from natural light using amber glassware. The sample was saponified with ethanolic potassium hydroxide and the carotene was extracted into hexane. The hexane was evaporated off to dryness and the carotene dissolved in mobile phase and quantified by HPLC with UV detection, against a calibration standard of known concentration.

 Folates were extracted from the samples using 0.1 M Potassium phosphate buffer and heating for 15 minutes at 100° C. The filtrate was diluted to a suitable level and treated with deconjugase enzyme. L-Ascorbic Acid was also added to prevent oxidation. This complex was incubated at 37° C for 4 hours. Using an Autodiluter, sample extracts were diluted to values within the calibrated range. Folic Acid Casei media was added to the diluted samples which were then covered with aluminium foil and sterilized at 121° C in an autoclave. The assay was inoculated with *Lactobacillus rhamnosus*, and was incubated overnight at 37° C. The concentration of folate in the sample was measured spectrophotometrically.

 The energy content of the snacks was calculated using the values for raw ingredients from Indian food composition tables (15).

*Biochemical measurements.* Within one hour from collection, venous blood was centrifuged at 20° C for 10 minutes (REMY, Pendleton, IN, USA). Depending on the assay, plasma or serum was pippetted into vials and kept on dry ice for up to eight hours before being transported to a –80° C freezer for storage until analysis. For vitamin C analysis, 0.3 ml plasma was added to 0.3ml of 10% metaphosphoric acid and stored at –80° C until analysis.

 A quantitative test kit based on solid phase enzyme linked immunosorbent assay was used to measure serum ferritin concentrations. The system used one anti-ferritin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme–linked antibodies. After incubation at room temperature for 60 min, the wells were washed with water to remove unbound antibodies. A solution of tetramethylbenzidine was added and incubated for 20 minutes, 2N HCl was added and the ferritin concentration was measured spectrophotometrically at 450 nm.

 For measurement of β-carotene,thawed subsamples of plasma were extracted with n-heptane in the presence of absolute ethanol, butylated hydroxytoluene (BHT) and α-tocopherol acetate (internal standard). The upper organic phase was evaporated nearly to dryness under vacuum, and was then re-dissolved in 250 μl of the mobile phase. Aliquots (50 μl) were then injected onto a 4 μ Waters C18 column which was preceded by a reduced stainless steel filter frit, to remove any particles. The mobile phase was acetonitrile 44%, methanol 44%, dichloromethane 12%, by volume, with added BHT at 10 mg/L. Β-carotene was measured at 450 nm using a Waters Millennium controlled HPLC system, with a photodiode array detector. Peak area response factors were obtained from semi-pure, commercially available carotenoids. These were then corrected to 100% purity, by means of their HPLC patterns, and from their absolute optical densities and known extinction coefficients (16).

 To measure retinol concentrations, 200 µl serum was added to 300 µl acetonitrile and vortexed for 90 seconds then centrifuged for 4 minutes. Supernatant was then separated and a 20 µl aliquot injected. Serum standards were prepared by adding known amounts of retinol to pooled serum to give the concentration required for each of their calibration curves. The limit of detection was 30 ng/ml and the limit of quantification 100 ng/ml (17).

 Ascorbic acid concentrations were assayed using a Roche Cobas Bio centrifugal analyser with fluorescence attachment. Ascorbic acid in the metaphosphoric acid stabilised plasma sample was converted to dehydroascorbic acid by ascorbate oxidase (Sigma, London). The resulting dehydroascorbate was coupled with o-phenylene diamine to give a fluorescent quinoxaline. The formation of this quinoxaline was linearly related to the amount of vitamin C in the sample, over the range 0-10 μg/ml (0-5μM). The validity of the fluorimetric assay procedure used was by cross-correlation with HPLC-based assays, and by vitamin C spiking experiments (18).

 Plasma folate concentrations were measured by microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus*. *Casei* and using Victor-2 (PerkinElmer Life Science, Turku, Finland) (19; 20).

 Total homocysteine (tHcy) was measured by fluorescence polarization immunoassay using Abbott homocysteine kits on Abbott Axsym system (Abbott Laboratories, Abbott Park, IL 60064 USA). The Axsym homocysteine assay is based on the Fluorescence Polarization Immumoassay technology. Bound homocysteine (oxidized form) is reduced to free homocysteine that is enzymatically converted to S-adenosyl-L-homocysteine which competes with labelled Fluorecein Tracer for sites on the monoclonal antibody molecule. The intensity of the polarized fluorescent light was measured by the Fluorescence Polarization Immumoassay optical assembly (21).

 Plasma C-reactive protein (CRP) was measured in order to assess levels of acute phase reactants in order to interpret the results of serum ferritin analysis. CRP was assayed using a high-sensitivity ELISA kit (United Biotech, Mountain View, CA, USA); Victor-2 (PerkinElmer, Turku, Finland). The coefficient of variation for the assay was <11%. The cut off value for active inflammation was 5 mg/L (22).

*Data Analysis.* Where data were normally distributed, means and standard deviations are presented, elsewhere the median and inter-quartile range is given. BMI was log transformed for regression analysis. The change in blood nutrient concentration over the 12 week period was calculated by subtracting the baseline (0wk) value from the 12 week (12wk) value. We performed paired t-tests to assess within-group differences between the mean 0wk and 12wk micronutrient concentrations. We also performed independent measures t-tests to assess differences between intervention groups in terms of the change (δ) over time (calculated as 12wk value - 0wk value). The effect of intervention group on change in nutrient concentration was assessed using univariate regression models. Although the women were randomised, we performed a multivariate regression adjusted for age, BMI, synthetic nutrient intake, compliance status, change in fruit intake frequency (12wk – 0wk), change in green leafy vegetable intake frequency (12wk – 0wk) with change in blood nutrient concentration as the dependent variable. Ferritin is an acute phase reactant, therefore high serum ferritin levels can be a result of inflammation rather than replete iron stores. Women with CRP concentrations >5 mg/L were excluded and the model was adjusted for CRP concentration. All statistical analyses were performed using the SPSS software package version 19 (SPSS Inc, Chicago, IL).

**RESULTS**

*Participant Characteristics.* The median (IQR) age of the women enrolled in the study was 20.5 (17.0, 26.0) years. Half were married and 81% were of Muslim faith. The majority (70%) of women were educated to secondary level with a further 9% receiving more than 10 years of formal education. A third were in paid employment at the time of the study. Mean (SD) height was 149.5 (5.6) cm and weight was 42.2 (8.8) kg. Median (IQR) BMI was 18.6 (16.3, 20.7) kg/m² indicating that a large proportion of the women in the study were underweight and chronically energy deficient. Thirty women reported taking synthetic micronutrient supplements during the study. Of these 18 took multiple-micronutrient supplements, 8 took iron and folate, and 4 took iron. Median (IQR) monthly family income was 4000 (3000, 5000) Rupees equivalent to approximately US$88 (66, 110) and the median (IQR) family size was 6 (5, 7) persons. Approximately 15% of women lived in ‘katcha’ style housing made from plastic sheeting, sticks and textiles. The remainder had ‘semi-pucca’ or ‘pucca’ walls and floors, made from cement with corrugated metal roofs. Most women (86%) used a shared pit toilet.

*Participant Flow.* Of the 222 women randomised, a total of 208 women with baseline blood measurements started receiving the intervention (figure 1). Of these, blood was collected at 12 weeks from 172 women and blood samples were available at both time points for 170 women. Compliance, defined as the proportion of women who consumed ≥3 snacks per week was 85% in the treatment and 90% in the control group. The median (IQR) weekly snack consumption of those in the final analysis was 5.0 (4.4, 5.5) supplements in the control group and 4.9 (3.8, 5.3) in the treatment group.

*Dietary Intake.* Intakes of green leafy vegetables and fruit, as reported in the interviewer-administered FFQ, increased between baseline and 12 weeks. Intakes of green leafy vegetables increased by a mean (SD) of 0.8 (2.2) servings per week in the control group from 1.3 (1.4) to 2.1 (2.0) servings per week and by 0.3 (3.2) in the treatment group from 1.4 (1.7) to 1.7 (2.6) servings per week, this difference did not reach statistical significance (p=0.144). There was no difference between groups in terms of the change in fruit intake (p=0.987), both groups increased by a mean (SD) of 1.7 (6.0) servings per week from 2.1 (3.1) to 3.9 (5.5) in the control group and from 2.0 (3.1) to 3.8 (5.1) in the treatment group.

*Blood Micronutrient Concentrations.* Median serum ferritin concentrations did not change in the control group and there was a small decrease in median concentrations in the treatment group (**Table 2**). When we removed cases where CRP levels were greater than 5000 ng/ml (n=28) and re-ran the analysis, there was little difference in median ferritin concentrations observed. Βeta-carotene concentrations increased in both groups but there was a significantly greater increase in concentrations in the treatment group. In both groups, median serum retinol concentrations decreased and there was no difference between groups. Plasma ascorbate concentrations did not change significantly. There was a decrease over the study period in both plasma folate and homocysteine but no difference between groups.

 Univariate analyses showed that being in the treatment group was associated with an increase in plasma β-carotene over the study period (**Table 3**). The effect size was approximately 10% of the mean baseline β-carotene concentration. For the other nutrients, treatment group did not predict changes in concentrations. The association between group and change in β-carotene concentrations remained when adjusted for age, BMI, intake of synthetic nutrients, compliance with the intervention and change in fruit and green leafy vegetable intakes (**Table 4**). For all nutrients the baseline values significantly predicted the change in concentrations and all associations were negative (data not shown).

**DISCUSSION**

This study aimed to assess the effect of consumption of a micronutrient-rich food-based intervention on the nutritional status of low income Indian women. We found that consumption of the treatment snacks for 12 weeks was associated with an increase in β-carotene concentrations relative to the control group. The mean difference in the change between groups was 49 nmol/L which was just over 10% of baseline β-carotene values. There was no significant effect of the intervention allocation on change in concentrations of any of the other micronutrients studied.

 In the UK, the effect of adding 85 g/d of raw watercress to the usual diet for 8 weeks was to increase beta-carotene levels by a mean of 100 nmol/L (23). Considering the quantity of green leafy vegetable in the intervention in the present study it would appear that the effect size is consistent with this finding.

 The change in the present study is considerably smaller than in studies where alterations to the entire diet pattern such as increasing daily intake of fruit and vegetables from two to ten portions per day have been implemented. Such interventions have achieved up to a 5-fold increase in β-carotene concentrations (24-26). It is questionable how sustainable such changes to diets would be in the Indian slum population.

 One explanation for the lack of effect seen with other micronutrients is that over three quarters of the women had retinol and folate concentrations above the cut offs for deficiency (data not shown). Therefore it is possible that the majority of the women in this population were not deficient in these nutrients. In addition the duration of the supplementation period may have been too short for retinol status to be altered. It was necessary for us to achieve a balance between resources, compliance with the protocol and observing an effect of the supplement on micronutrient status. Further, in the case of vitamin C, the content in the snacks was low, probably due to the cooking process. There was some experimentation with different methods of cooking and preparing the snacks so as to preserve vitamin C content but the most acceptable method to the women was shallow frying and it was important to achieve adherence to the supplementation protocol.

 We assessed the possible impact of inflammation on ferritin status using CRP concentrations but this did not alter the lack of association with treatment allocation. The snacks contained on average 6.2 mg of iron per serving. However, it is likely that inhibition of iron uptake due to frequent consumption of phytate-containing cereal based foods or tea containing polyphenols, and polyphenols in the green leafy vegetables in the snacks themselves, meant there was insufficient bio-available iron in the snacks to improve iron status.

 A study in a small group of healthy young females in Pune, West India compared the short term effects of consumption of a green leafy vegetable meal with a standard meal without green leafy vegetables (27). No difference in plasma β-carotene or vitamin C concentrations between the two groups was observed 4 hours after the meal. However, after a 3 week intervention period constituting daily supplementation with 100 g cooked green leafy vegetables and 10 g oil, there was a significant increase in plasma concentrations of both nutrients. The authors concluded that intake of 100 g green leafy vegetables per day plus 10 g oil could be an effective strategy for improving micronutrient status in young Indian women. The treatment snacks in the present study contained approximately 25 g green leafy vegetables per serving, it is possible that there is a ‘threshold nutrient intake’ above which a change in nutrient concentration would be observed. For example, consumption of four snacks per day may have led to increases in concentrations of the other nutrients and a more marked increase in β-carotene concentration in the treatment group.

*Strengths and limitations of the study.* Prior to the present study, there was a paucity of data on the effect of long-term food based interventions on the nutritional status of women in low-income settings. We used a randomised controlled design but as with many food-based intervention studies it was not possible to blind the intervention. It is unlikely that this would influence the findings given that the outcomes were objectively assessed and the group allocations were not known to the laboratory staff who made the nutrient concentration measurements. A strength of the study is that all of the participants started and completed the intervention at the same time, thus creating an internal control for seasonal changes in food intakes.

 The sample size calculation was based on serum retinol concentrations as this was the nutrient with available data in the Indian population. Based on previously published data, we over-estimated the proportion of women who would be retinol deficient and it is possible that the study was underpowered for other nutrients for which no status data in this population were available.

 It is possible that the women changed their habitual diet as a result of being enrolled in the study, it is also conceivable that women in the treatment group adjusted their consumption of green leafy vegetables because they were aware that it was in the snack. Women in the control group may have done the opposite thus negating the effect of the snacks on micronutrient status. Our data suggest that this did happen to a certain extent in the current study and the effect size we have shown in relation to β-carotene concentrations may be an underestimation. A similar phenomenon was observed in the UK school fruit scheme whereby children who received fruit at school were given less fruit at home so an intervention designed to increase children’s intake of fruit by 1 portion per day effected only a 0.5 portion per day increase (28) and this may have been due to the fact that parents assumed that because children had eaten fruit at school they required less at home (29). This phenomenon represents an important challenge when designing food-based interventions.

 In conclusion, this study indicates that small changes in intakes of fruit, green leafy vegetables and milk in a food based snack intervention can lead to increased levels of circulating β-carotene in this population.

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**CONFLICT OF INTEREST**

None.

**AUTHORSHIP**

The authors’ contributions are as follows: SK contributed to the conception and design of the study, data collection and analysis, and wrote the manuscript; CF and BM contributed to the conception and design of the study and reviewed/edited the manuscript; HC and DT contributed to the design of the study, data collection and reviewed/edited the manuscript; SS,NB,MG and RP contributed to the design of the study and reviewed/edited the manuscript; DB, RM, SY contributed to data collection and biochemical analysis and reviewed/edited the manuscript.

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**TABLE 1: Mean micronutrient content of treatment and control snacks.**

|  |  |
| --- | --- |
|  | Nutrient |
|  |  | Energy *mJ* | Iron *mg* | β-carot *μg* | RE *μg* | Ascorbate *mg* | Folate *μg* |
| Treatment snacks |  |  |  |  |  |  |  |
| Nutrient content values/100g  |  | 0.66 | 6.2 | 1367 | 228 | 3.5 | 97 |
| %UK EAR per serving\* |  | 8 | 35 |  | 37 | 9 | 42 |
| Control snacks |  |  |  |  |  |  |  |
| Nutrient content values/100g  |  | 0.36 | 3.6 | 23 | 4 | 0 | 26 |
| %UK EAR per serving\* |  | 4 | 11 |  | <1 | 0 | 6 |

\*Percentage of the UK Estimated Average Requirement achieved in one daily serving; RE=retinol equivalents; β-carot, β-carotene;

TABLE 2: Median (IQR) blood nutrient concentrations at baseline and at 12 weeks of supplementation, and the change in concentrations, in treatment and control groups.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Control |  |  | Treatment |  |  |
|  | N | 0wk | 12wk | p\* | Change(12wk-0wk) | N | 0wk | 12wk | p\* | Change(12wk-0wk) | p† | δ (CI) |
| Ferritin (µmol/L) | 83 | 6.4 (4.6,11.0) | 6.4 (5.0,11.5) | 0.238 | 0.0 (-1.0,2.5) | 77 | 6.4 (5.0,11.8) | 7.6 (5.0,10.7) | 0.131 | -0.4 (-2.5,1.0) | 0.065 | -1.51 (-3.11, 0.09) |
| Ferritin (µmol/L)‡ | 69 | 6.2 (4.6,11.0) | 6.4 (4.8,11.5) | 0.387 | 0.0 (-1.05,2.1) | 66 | 6.4 (5.0,12.0) | 7.6 (5.0,11.0) | 0.189 | -0.3 (-2.6,1.1) | 0.173 | -1.26 (-0.56,3.07) |
| β-carotene (nmol/L) | 85 | 390 (305,470) | 440 (340,540) | 0.009 | 40 (-5,115) | 80 | 385 (323,470) | 470 (380,610) | <0.001 | 75 (23,158) | **0.020** | **48.7 (7.4,89.7)** |
| Retinol (ng/ml) | 83 | 409 (326,490) | 395 (324,476) | 0.278 | -10.4 (-91.2,44.2) | 79 | 378 (297,484) | 358 (291,474) | 0.387 | -17.7 (-94.4,65.0) | 0.829 | 4.29 (-34.76,43.33) |
| Ascorbate (µmol/L) | 66 | 12.5 (8.8,20.3) | 14.6 (9.6,27.0) | 0.033 | 1.5 (-2.3,8.3) | 70 | 12.7 (8.6,22.3) | 17.0 (9.9,29.5) | 0.276 | 0.58 (-3.71,8.01) | 0.652 | -1.15 (-6.18,3.88) |
| Folate (nmol/L) | 80 | 14.1 (9.0,21.4) | 13.1 (9.2,18.4) | 0.085 | -0.4(-5.5,4.0) | 77 | 13.3 (9.9,19.6) | 13.3 (9.2,17.5) | 0.105 | -0.6 (-3.9,2.4) | 0.757 | -1.35 (-9.99,7.28) |
| Homocysteine (µmol/L) | 80 | 11.6 (9.5,13.9) | 10.4 (8.8,13.4) | 0.118 | -0.5 (-2.5,0.5) | 76 | 11.3 (8.7,14.6) | 10.6 (8.6,13.4) | 0.022 | -0.3 (-2.8,1.0) | 0.738 | 0.33 (-1.61, 2.27) |
| Haemoglobin (g/dL) | 88 | 11.4 (10.3,12.5) | 11.9 (10.6,12.6) | 0.027 | 0.1 (-0.3,0.8) | 82  | 11.4 (10.5,12.2) | 11.7 (10.8,12.3) | 0.095 | 0.1 (-0.3,0.5) | 0.638 | 0.07 (-0.22,0.36) |

\*P value relates to the difference in mean values at 0 weeks and 12 weeks. †P value relates to t-test for difference in the 12wk-0wk values between treatment groups. ‡Excluding women with CRP>5000ng/ml. δ, difference in the change (12wk-0wk) between treatment groups (treatment – control).

**TABLE 3: Summary of univariate models with treatment group as the predictor variable and change (δ) between baseline and 12 week measurements as the outcome variable (12wk-0wk).**

|  |  |  |  |
| --- | --- | --- | --- |
| Blood concentrations (12wk-0wk) |  | 95% Confidence Interval |  |
|  | B | Lower | Upper | p |
| Ferritin (ng/ml) | -1.51 | -3.10 | 0.09 | 0.064 |
| Β-carotene (nmol/L) | 49.0 | 10.0 | 90.0 | **0.020** |
| Retinol (ng/ml) | 5.76 | -33.14 | 44.67 | 0.770 |
| Ascorbate (µmol/L) | -1.18 | -6.17 | 3.81 | 0.641 |
| Folate (nmol/L) | -1.39 | -9.97 | 7.18 | 0.749 |
| Homocysteine (µmol/L) | 0.32 | -1.60 | 2.25 | 0.741 |
| Haemoglobin (g/dL) | -0.07 | -0.36 | 0.22 | 0.638 |

B, Beta coefficient.

**TABLE 4: Summary of multivariate models with change (δ) in micronutrient concentration (12wk-0wk) as the outcome.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Ferritin (ng/ml) | Β-carotene (µmol/L) | Retinol (ng/ml) | Ascorbate (µmol/L) | Folate (nmol/L) |
|  | B (CI) | p | B (CI) | p | B (CI) | p | B (CI) | p | B (CI) | p |
| Group (0,1)\* | -1.39 (-3.32,0.54) | 0.157 | 49.64 (7.05,92.22) | **0.023** | 2.90 (-38.00,43.80) | 0.889 | -1.31 (-6.54,3.91) | 0.620 | -2.85 (-11.55,5.84) | 0.518 |
| Age (years) | 0.06 (-0.11,0.22) | 0.492 | 1.89 (-2.01,5.80) | 0.340 | -0.14 (-3.96,3.68) | 0.942 | 0.01 (-0.48,0.49) | 0.978 | -0.15 (-0.94,0.65) | 0.712 |
| BMI (kg/m2) | -0.01 (-0.33,0.31) | 0.956 | -3.57 (-10.03,2.89) | 0.277 | -1.39 (-7.57,4.80) | 0.658 | -0.05 (-0.84,0.74) | 0.905 | -0.59 (-1.91,0.73) | 0.379 |
| Synthetic Nutrient Intake at 12wks\*\* | 3.45 (-0.35,6.56) | 0.030 | -21.83 (-87.29,43.63) | 0.511 | 31.85 (-33.68,97.39) | 0.338 | 4.24 (-4.03,12.52) | 0.422 | 16.28 (2.54,30.02) | **0.021** |
| Compliance Status\*\*\* | -1.99 (-7.4,3.39) | 0.465 | -15.35 (-115.29,84.58) | 0.762 | 21.33 (-89.18,131.84) | 0.704 | -8.55 (-19.92,2.82) | 0.139 | -6.48 (-27.06,14.11) | 0.535 |
| Δ Fruit intake (frequency) | 0.14 (-0.07,0.35) | 0.200 | 2.38 (-1.51, 6.27) | 0.229 | -0.16 (-3.81,3.50) | 0.932 | -0.15 (-0.62,0.32) | 0.526 | 0.18 (-0.62,0.98) | 0.650 |
| Δ GLV intake (frequency) | -0.06 (-0.70,0.58) | 0.847 | 5.15 (-3.85,14.16) | 0.260 | -0.50 (-9.43,8.44) | 0.913 | 0.51 (-0.76,1.77) | 0.430 | -0.70 (-2.84,1.44) | 0.519 |

B, Beta coefficient. CI, 95% Confidence Interval. Δ, change in frequency of intake (12wk – 0wk) \*Treatment group; Control=0, Treatment=1, \*\*Synthetic nutrient intake; No=0, Yes=1, \*\*\*Compliance status; No=0, Yes=1

**Figure 1: Participant flow chart**