12 Developing Micronutrient-rich Snacks for Pre-conception and Antenatal Health: the Mumbai Maternal Nutrition Project (MMNP)

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Abstract

Observational and trial data suggest that poor maternal micronutrient status as a result of poor dietary quality before and during pregnancy impairs fetal growth and development. This chapter describes the development of palatable food supplements produced from locally available vegetarian foods that improve the quality of the diet of young Indian women living in Mumbai slums.

A vehicle in the form of a cooked snack food that could be distributed daily was developed to provide the women with supplementary green leafy vegetables (GLVs), fruit and milk. The target nutrient content of the snack was defined based on intake data from the study population and the UK Estimated Average Requirement (EAR). The snack was analysed to measure these target nutrient levels and palatability was assessed.

Several approaches were used to deliver the amount of GLVs, fruit and milk that were considered sufficient to have an impact on the women's nutritional status. A vehicle was developed that contained these micronutrient-rich foods and was palatable and acceptable to the women. Some of the target micronutrient levels were achieved using combinations of fresh GLVs, dried fruits and milk powder. Mean micronutrient levels of the final product (per serving) were: β-carotene 123 retinol equivalents; folate 68 μg; riboflavin 0.14 mg; iron 4.9 mg; calcium 195 mg; vitamin B_{12} 0.24 μg. These values are between 12% and 43% of the UK EAR. To date, target vitamin C levels have not been achieved.

It is possible to develop palatable, culturally acceptable and safe micronutrient-rich food supplements using a low-tech approach and locally available fresh and dehydrated ingredients.

Key words: micronutrient, food supplement, green leafy vegetables, fruit, milk, India

Introduction

Suboptimal maternal micronutrient status during the periconceptional period, and during pregnancy itself, adversely affects fetal growth and the development of all body tissues, impairing the subsequent health of the baby, child and adult (1). Birth weight is a crude measure of fetal development, but nevertheless it has been shown to be associated

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with long-term health outcomes including cardiovascular disease and diabetes (2). According to data from the third Indian National Family Health Survey (NFHS3) (3), in 2005 almost 22% of neonates in India weighed less than 2.5 kg. A more recent estimate published in 2009 by the United Nations Children's Fund (UNICEF) is 28% (4). It has been documented that babies born small are more likely to be stunted in childhood (5–7), which in females is a risk factor for low birth weight in the next generation and for both males and females is associated with lower adult income (8). Estimates of the prevalence of stunting in India range from 38% for children under 5 years of age (UNICEF) (4) to 45% for children under 3 years (NFHS3) (3). Stunting, wasting and underweight for age at 3 years are all more prevalent among children of lower socio-economic status (3).

The Mumbai Maternal Nutrition Project (MMNP) is a randomized controlled trial investigating the effect of providing women with a micronutrient-rich food for consumption before and throughout pregnancy on infant outcomes, including size and weight at birth, infant mortality, childhood growth, cognitive development and cardiovascular risk. The trial participants are married women of childbearing age who are intending to become pregnant and are living in a slum community in the city of Mumbai, India. The trial was launched in January 2006 and is due to run until 2011. It was approved by the research ethics committee of the Nair Hospital, Mumbai, and is on the International Standard Randomized Controlled Trial Register (ISRCTN 62811278).

The MMNP was inspired by the Pune Maternal Nutrition Study (PMNS) which was carried out between 1994 and 1996. The PMNS was an observational study in a rural Indian population in which women were interviewed about their dietary habits twice during pregnancy using a food-frequency questionnaire (FFQ) developed specifically for the population. The women were then followed up at pregnancy and detailed anthropometry of the baby was carried out within 72 h of birth. The results showed that mothers (n 633) with higher self-reported intakes at 28 weeks' gestation of green leafy vegetables (GLVs), fruits and milk delivered fewer low-birth-weight babies (9).

The MMNP was subsequently designed to investigate whether this relationship was causal, i.e. whether consumption of these foods by the mother before and during pregnancy positively affected the development of the fetus. The intervention is based on locally available foods, rather than synthetic nutrients. This approach requires that the women consume the foods on a regular basis prior to and during pregnancy. It is intended that the intervention is an addition or supplement to the women's daily intake rather than a replacement for any of the foods she would habitually eat. It was therefore decided that the supplement would take the form of a snack food similar to those available locally from street-side stalls and be made available to women at a time of day when they would not usually be eating a meal. Once enrolled in the study, the women are asked to visit a distribution centre within walking distance of where they live six days per week in order to receive the supplement. The woman's attendance at the centre and consumption of the supplement are recorded by health workers on each visit. Pregnancies are identified by recording the women's last menstrual period date. Women who become pregnant are asked to continue to eat the supplement until delivery.

The trial has an intervention and a control arm. The control supplement contains vegetable ingredients of relatively low micronutrient content that were not associated with birth outcomes in the PMNS. Blinding is not possible in this study but in order to mask the true nature of the intervention the women are randomized to one of four groups. On a given day, four different snacks are sent to the field, two varieties of intervention and two varieties of control.

This chapter describes the stages of development of the intervention supplement and the degree to which the following requirements have been achieved:

1. Produced from locally available foods.
2. Acceptable to vegetarians.
3. Contains target levels of several 'marker' micronutrients.

4. Possible to prepare daily in large quantities by local staff in a 'low-tech' kitchen.
5. Palatable enough to be eaten daily over a period of months.
6. Microbiologically safe (not containing salmonella, *Escherichia coli*, coliforms or unacceptable levels of mould).
7. Nutritionally safe (not exceeding UK safe upper limits for any nutrient) (10).

The project started with a two-year pilot study (2004–2005) in the Shetanchowki area of Mumbai, based in the Streehitakarini Health Centre. The main trial started in 2006 in Bandra, based at the Centre for the Study of Social Change, and as of April 2010 has recruited approximately 5000 women.

Methods

It was decided that the supplement would take the form of a snack food similar to those available locally from street-side stalls and be made available to women at a time of day when they would not usually be eating a meal. Nutrient content, acceptability, safety, cost and availability of ingredients, manpower and cooking facilities have been considered when developing the snacks. There have been four chronological stages of snack development: pilot study; main trial 1; main trial 2; and main trial 3.

Target nutrient content

The starting point for setting the target nutrient content of the snacks was information from the PMNS which collected data on women's habitual food intake using a 111-item FFQ (9). We estimated average intakes of 'marker' nutrients: β-carotene, riboflavin, folate and vitamin C. The amount of nutrient that would increase daily intakes of the 'marker' nutrients above the 75th centile of intakes of the women in the PMNS was then calculated (Table 12.1). Because it was anticipated that MMNP participants would be more likely to attend on alternate days (i.e. three days per week) rather than six days per week, the target nutrient content was set at double the amount that would move intake to the 75th centile of the PMNS. This amount was found to be approximately equal to one-third of the UK Estimated Average Requirement (EAR) for riboflavin and folate (11).

At each stage of the trial, samples of the snacks were tested for nutrient content. Homogenized and frozen snacks were flown to the UK on dry ice and analysed at a commercial laboratory (Eclipse Scientific Group, Cambridge). Reversed-phased high-performance liquid chromatography was used to test for riboflavin, β-carotene and vitamin C; vitamin B_{12} content was analysed using surface plasmon resonance inhibition assay; folic acid was analysed by bioassay

Table 12.1. Amount of nutrients consumed by women in the PMNS and the target nutrient content of the MMNP supplement.

	β-Carotene (RE)	Riboflavin (mg)	Folate (μg)	Vitamin C (mg)
Women's median daily intake during pilot study	600[a]	0.65	126	21
75th centile of intake in PMNS	654[a]	0.82	164	23
Target nutrient content of snack	108[a]	0.34	76	4
UK EAR (11)	500[a]	1.2	250	25
Safe Upper Limit (where applicable) (10)	1166[b]	–	–	–

PMNS, Pune Maternal Nutrition Study; MMNP, Mumbai Maternal Nutrition Project; RE, retinol equivalents; EAR, Estimated Average Requirement.
[a]Figures relate to all sources of retinol.
[b]Figures relate to β-carotene only.

(*Lactobacillus rhamnosus*); and all minerals were analysed by inductively coupled plasma-atomic emission spectrometry.

Formulation

We initially considered supplying the food in the form of a milk drink, a piece of raw fruit and an ordinary cooked GLV preparation. However, this was not feasible in the context of the trial, for a number of reasons. First, the daily purchase of all these fresh ingredients was too costly in terms of staff time. Second, it was not possible to deliver these foods in a palatable state, or to maintain microbiological safety, during their distribution throughout the large slum area. Third, it would have been difficult to record the women's intake, in a simple way, with the foods in this form. Finally, it was clearly impossible to make the intervention and control supplements appear similar using this approach. We therefore decided to combine the three ingredients and to make them into cooked snacks, similar to street snacks, like samosas, widely available in Mumbai.

During the pilot study phase, it was not possible to purchase and prepare sufficient quantities of fresh ingredients for the snacks due to manpower constraints. Initial formulations therefore contained dehydrated, powdered GLVs, fruit and milk. A Mumbai-based commercial company supplied vegetable and fruit powders prepared using a novel technique of room-temperature drying. These powders have superior smell and flavour compared with heat-dried powders and nutrient retention is maximized. The use of powders allowed the inclusion of greater quantities of the GLV, fruit and milk in the limited volume available. They were combined with other 'binding' or 'covering' ingredients such as chickpea flour or semolina and seasoned with local spices to give a product which resembled a food like a samosa or patty.

Product development

The recipes for the snacks were initially developed by the project nutritionists, experimenting on a small scale at home in their kitchens. Preparation of the snacks was then scaled up with the installation of a large project kitchen, staffed by 19 men and women and equipped with a range of basic kitchen facilities (including a large gas stove, oven, chilled storeroom and stainless steel preparation surfaces). Development of new recipes (to avoid monotony for the women) and the introduction of more palatable formulations have been an ongoing process throughout the pilot study and the main trial.

Choice of specific green leafy vegetable

The choice of GLV to be added to the supplements was initially based on the availability of the dehydrated powders and the opinions of project staff as to acceptability. In early 2007, the dehydrated powders of ten different GLVs (radish leaf, red amaranth, fenugreek, green amaranth, coriander, colocasia, drumstick leaf, onion stalk, shepu, spinach and curry leaf) were analysed by a UKAS accredited laboratory (Eclipse Scientific Group) for micronutrient content. The powders were also analysed for polyphenol (Global Analytical Services, Heidelberg, Germany) and oxalate content (Lincoln University, Canterbury, New Zealand) (12). Polyphenols and oxalates are considered 'anti-nutrients' because they inhibit absorption of minerals, specifically iron and calcium, respectively (13,14). The dehydrated powders were crudely ranked according to nutrient and 'anti-nutrient' content; those with the lowest overall score being the most nutritious and containing the least anti-nutrient.

Stages of development

As the study progressed a series of major changes was made to the snacks (Table 12.2). These were mainly to improve the nutrient quality and palatability, the latter having an impact on participant compliance. First, the amount of GLV powder added to the snacks was reduced; this was to make the snack more palatable (large amounts of dried GLV made

Table 12.2. Ingredients, mean nutrient composition and mean percentage contribution to nutrient requirements of the supplements at each stage of the trial.

	Pilot study	Main trial 1	Main trial 2	Main trial 3
	Dry GLV, fruit powder	Reduced GLV powder	GLV powder + fresh GLV	Fresh GLV
	Jan 2003 to Jun 2005	Jan 2006 to Oct 2006	Oct 2006 to Jun 2007	Jun 2007 to present
Ingredient				
Dry GLV powder (g)	10	7.5	3.8	0
Milk powder (g)	8	16	12	12
Fruit powder (g)	4	4	4	0
Fresh GLV (g)	0	0	29	30
Dried fruit (g)	0	0	0	4
Micronutrient content per supplement				
β-Carotene (RE)	282	114	200	123
Riboflavin (mg)	0.58	0.20	0.21	0.14
Folate (μg)	135	26.0	50.8	67.5
Vitamin C (mg)	1.6	<1	0.5	1.5
Vitamin B_{12} (μg)	0.61	0.64	0.58	0.24
Calcium (mg)	298	210	275	195
Iron (mg)	5.46	6.85	5.90	4.90
Macronutrient content per supplement				
Energy (kJ)	795	741	703	611
Energy (kcal)[a]	190	177	168	146
Protein (g)[a]	7.0	7.3	6.9	6.4
% of target (% of EAR[b])				
β-Carotene	261 (56)	105 (23)	185 (40)	114 (25)
Riboflavin	170 (48)	59 (17)	62 (18)	41 (12)
Folate	177 (54)	34 (10)	67 (20)	89 (27)
Vitamin C	40 (6)	<1 (<1)	13 (2)	38 (6)
Vitamin B_{12}	(48)[c]	(51)[c]	(46)[c]	(6)[c]
Calcium	(48)[c]	(34)[c]	(44)[c]	(31)[c]
Iron	(48)[c]	(60)[c]	(52)[c]	(43)[c]
Compliance (% of participants consuming whole rather than half supplement)	Not measured	64	72	89

GLV, green leafy vegetable.
[a]Macronutrient content calculated using Indian Food Tables (16).
[b]UK Estimated Average Requirement during pregnancy (11).
[c]No target was set for these nutrients.

it taste bitter) (Table 12.2, main trial 1). Next, 50% of the GLV powder was substituted with fresh GLVs (Table 12.2, main trial 2). There were, however, other reasons for some of the changes; a problem with rat infestation on the premises of the commercial dehydrated powder suppliers forced a complete change to the use of fresh rather than dried GLVs and dried fruit rather than dried fruit powder. The final and current formulation being used in the trial is shown in Table 12.2 (main trial 3).

Assessment of the acceptability and safety of snacks

New snack recipes were tested for palatability by project staff and small panels of local women before being distributed to the field. In addition to this anecdotal approach, acceptability was assessed more objectively using data recorded daily by health workers on the consumption of the snacks. The proportions of women attending the distribution centre and consuming the whole snack (recorded as '1'), at least half but not the whole snack (recorded as '0.5') or less than half (recorded as '0') were calculated and used to assess the acceptability of each recipe (Table 12.2, compliance). Microbiological testing for the presence of coliforms in snacks was performed during the pilot study at a Mumbai food safety laboratory. All snacks are prepared and cooked fresh every day and leftovers discarded.

Cost

The costs of the ingredients, staff wages, cooking fuel and packaging were used to calculate the unit cost of the snacks. This was compared with the cost of the UNICEF multiple micronutrient tablet (15).

Food intake

We assessed the baseline food intake of the women using a 213-item FFQ which was administered by trained interviewers to women at enrolment. The reference period was the most recent week. The questionnaire covered the vast majority of foods that the women were likely to eat and provided detailed information on the amount of fruit, vegetables and milk products consumed. The increase in intake of these foods as a result of consumption of the snack was then calculated with reference to the baseline median intake.

Results

Nutrient content/acceptability

Table 12.2 shows the average nutrient content of the snacks broken down by trial stage. The first version of the snack (Table 12.2, pilot study) was rich in micronutrients with the exception of vitamin C. However, the dry GLV powder made the snacks dense and difficult to eat, and some women ceased to comply with daily consumption. Reducing the GLV powder and increasing the milk powder content (Table 12.2, main trial 1) improved compliance but led to an unacceptable drop in certain nutrients, particularly folate. The best overall combination of palatability, compliance, nutrient content and appearance has been achieved by complete substitution of the GLV powder with fresh GLVs (Table 12.2, main trial 3). The target of raising the daily intake to that of the 75th centile of the PMNS women is met for the majority of the 'marker' nutrients. A significant proportion of the UK EAR is also met for several nutrients. None of the snacks has achieved target vitamin C levels and vitamin B_{12} levels were low.

Choice of green leafy vegetable

The results of the GLV analysis are shown in Table 12.3. The GLVs with the lowest overall score were the most favourable. If the results are interpreted including all of the nutrients in Table 12.3, curry leaves and spinach have the lowest content of bioavailable nutrients and radish leaves and red amaranth have the highest.

Since the results of these analyses were available in mid-2007, the choice of GLV added to the supplements has been based on these results where availability and cost have allowed. The acceptability according to the project team has also been taken into account. For example, despite having a favourable nutrient profile, fenugreek was removed from the snacks as the women did not like the bitter taste of this GLV.

Safety

All microbiological tests for coliforms were negative in the pilot study. These were not repeated during the main trial. None of the snacks exceeded the safe upper limit for β-carotene content.

Table 12.3. Nutrient and anti-nutrient composition of GLV dehydrated powders and ranking according to nutrient content.

GLV	Nutrient content per 100 g									Nutrient score[a]	Anti-nutrient content		Anti-nutrient score[b]	Overall score	Overall rank[c]
	Ca (g)	Fe (mg)	Mg (g)	Zn (mg)	Vitamin B_{12} (µg)	Vitamin B_2 (mg)	RE (µg)	Vitamin C (mg)	Folate (µg)		Soluble oxalate (% of total oxalate)	Polyphenol (mg/kg)			
Radish leaf	2.61	57.40	0.90	5.92	2.40	1.52	7250	51.1	800	38	0.00	10.90	2	40	1
Red amaranth	2.79	212.00	1.66	4.60	1.40	0.80	6200	35.6	1300	38	43.90	16.50	10	48	2
Fenugreek	1.33	129.00	0.34	2.50	1.10	2.02	3820	186.0	770	56	0.00	15.50	3	59	3
Green amaranth	2.71	97.00	2.16	4.00	0.93	0.33	7900	10.6	890	51	46.50	15.90	10	61	4
Coriander	1.10	108.00	0.39	4.40	2.90	1.09	6100	58.0	730	60	0.00	25.90	5	65	5
Colocasia	2.12	46.50	0.52	3.10	<0.2	1.55	8320	15.9	680	58	25.20	10.30	7	65	6
Drumstick	3.02	58.90	0.64	1.90	1.10	1.29	2480	167.0	540	63	0.00	17.80	4	67	7
Onion stalk	1.68	95.30	0.40	1.70	3.70	0.79	3180	51.0	550	69	0.00	9.04	1	70	8
Shepu	1.63	102.00	0.49	3.10	1.20	0.34	2920	105.0	770	64	0.00	26.20	6	70	9
Spinach	1.07	87.00	1.17	5.10	1.00	1.43	5280	13.3	440	70	94.60	8.51	9	79	10
Curry leaves	2.80	43.10	0.60	2.90	1.60	0.30	1460	45.5	150	78	0.00	41.00	7	85	11

GLV, green leafy vegetable; RE, retinol equivalents.

[a]Nutrient score = sum of the ranks for each nutrient. Lower score represents GLV with a greater nutrient content.

[b]Anti-nutrient score = sum of the ranks for each anti-nutrient. Lower score represents a GLV with a lower anti-nutrient content.

[c]Overall rank: 1 is most favourable nutrient and anti-nutrient profile.

Cost

The average unit production cost of snacks made using dehydrated leaves (main trial 1) was 13 Indian rupees (approximately US$0.33). This cost was reduced to 5 rupees (US$0.13) for the snacks made with fresh GLVs. These prices are comparable to the cost of similar 'street' snack foods that women consume in this part of India. The unit cost is higher than that of the UNICEF multiple micronutrient tablet, which is approximately US$0.02 per daily dose (15).

Food intake

The baseline frequency of intake of GLVs and fruit in this population was found to be very low: median (interquartile range, IQR) of 1.1 (0.57–1.29) and 0.3 (0.15–0.6) servings/day, respectively. Milk was frequently consumed, the median (IQR) frequency being 2.1 (2–3), but in approximately 80% of cases the serving was that added to tea and therefore a relatively small quantity (approximately 30 ml/serving).

In this population, one serving (and one FFQ portion size) is approximately 30 g. Consumption of the snack increased median intake of fruit and GLV by approximately 34 g/day (or 113%). Cow's milk is approximately 88% moisture (16), so 12 g of milk powder is equivalent to approximately 100 ml of milk. The median frequency of milk intake was two portions of 30 ml/day. Consumption of the snack therefore increases daily median milk intake by approximately 167%.

Discussion

We have demonstrated that it is possible in the context of an urban community in India to develop palatable micronutrient-rich snacks that young pre-pregnant and pregnant women will eat on a daily basis. This has been achieved using a variety of locally available foods and employing local staff. The potential advantages of a food-based supplement made using low-tech methods from local ingredients over synthetic nutrients, such as implemented in the MMNP, are important and should be emphasized. It is likely that such an approach will be more acceptable and sustainable in the long term as a means of improving health and nutrient status. It also provides social enterprise opportunities within the community (including for agriculturalists and kitchen staff), thus benefiting local people financially.

While the nutrient content of such foods is unlikely to exceed that of pharmaceutical interventions, it was possible to achieve target micronutrient levels for some of the nutrients using locally available 'food' ingredients without the addition of any synthetic micronutrients. A synthetic micronutrient preparation requires selection of particular nutrients, and it is not always known which nutrients should be included. It is likely that there are nutrients (e.g. essential fatty acids) and phytochemicals (bioactive non-nutrient plant compounds, e.g. flavonoids) in such foods that are important in disease prevention (17,18) and are not present in pharmaceutical interventions. No data are available on the bioavailability of the nutrients present in the snacks or changes to the health or nutrient status of the women. It will be important to examine the longer-term effects of daily supplementation on the micronutrient status of people in this community. The baseline GLV and fruit intake of the women was found to be very low and while the snacks may not be providing the full recommended daily intake of fruit and vegetables, the increase as a result of consuming the snacks is substantial. The aim of the supplementation programme is to positively shift the distribution of nutrient intake to ensure that all women are achieving a nutrient intake that is associated with better health outcomes for their children. The aim is not to change the distribution of intake such that all women are receiving the reference nutrient intake (equivalent to two standard deviations above the average requirement). We believe that this public health approach is likely to be more acceptable to the women and more sustainable for their community.

The unit cost of the snack in the MMNP was substantially more expensive than that of a micronutrient tablet. However, as pointed out by Gopalan (19), in countries such as India where micronutrient deficiencies exist as a result of a poor dietary quality, increasing the consumption of micronutrient-rich foods in a manner which is sustainable to the community is likely to be more effective than giving out tablets that are manufactured in high-income countries. The commercial viability or marketability of the snack has not been tested at this stage. The average cost of a similar type of street food snack in this community would, however, be at least as much or slightly more than the cost of producing our snack, so we are confident of the viability of scaling up the project if the trial shows that there is a positive effect on birth outcomes.

In our experience, this method of supplement development takes a substantial amount of time. The length of time is dependent on a number of factors. Each recipe requires experimentation in the kitchen by project staff with a prescribed set of ingredients. The next stage is for taste panels to approve the snack. They must then be analysed for nutrient content. For the current study, it was not possible to find a suitable laboratory in India for nutrient analysis to be carried out. This necessitated transportation of samples to the UK, adding to the development time. Finally the 'acceptability' of the snacks to the women participating in the study had to be assessed to ensure that they would consume them on a regular basis.

Perishability of the snacks is a potential problem that could limit the adoption of this approach on a large scale. The snacks in the trial are cooked and eaten on the same day. Another is lack of availability of some of the 'active' ingredients at certain times of the year (e.g. during the monsoon months, fewer GLVs are available, and those that can be obtained tend to be the less nutritious ones). We believe that the problems of poor availability or contamination should not be insurmountable, and have plans to re-introduce some dried leaf into the intervention supplements. Dried GLV and fruit powders offer one method of long-term storage of micronutrient-rich foods that could be viable at community level in India. Future plans include the development of a supplement with a longer shelf-life. This will enable the kitchen staff to plan for holidays, festivals and times when ingredients are in short supply.

To date, we do not know the effects of supplementation on any of the outcomes in the trial, which include maternal nutrient status, birth weight and infant body composition. It is expected that the trial will be completed in 2011, and that data will be available from 1600 pregnancies.

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