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ADDRESSING IRON BIOAVAILABILITY: CORE STRATEGY TO ACHIEVE ANAEMIA MUKHT BHARAT

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Introduction

Iron is the fourth most abundant element (O, Si, Al, Fe) and second most abundant metal in the earth's crust. The chemical formula, atomic number and atomic weight of iron are Fe, 26, 55.65 respectively. It is a vital nutrient required for a wide variety of essential cellular processes and occupies a unique place in health and well-being of humans as it and, as such. The element holds this central position by virtue of its facile redox chemistry and the high affinity of both redox states (reduces ferrous iron Fe^{2+}/oxidised ferric iron Fe^{3+}) for oxygen. These same properties also render iron toxic when its redox-active chelatable 'labile' form exceeds the normal binding capacity of the cell.

Under aerobic physiological conditions, iron is present mostly in its oxygenic ferric, virtually insoluble form, with 10^{-9} M concentration (0.558 µg/L) of Fe^{3+} at pH 7. Iron is the major hemopoietic factor and acts as a catalyst in redox reactions by donating or accepting electrons and participates in free radical chemistry by donating electrons to oxygen. The redox potential of Fe^{2+}/Fe^{3+} varies between ~–0.5 V and ~+0.6 V.

In the human body iron once absorbed is recycled and retained as there is no physiologic mechanism for its excretion. Therefore, iron homeostasis is closely regulated via intestinal absorption. About 1 mg of iron is lost per day by males and 1.5 mg by menstruating females, majorly through blood and mucosal epithelial cell loss. Thus, solubility and thereby absorption or bioavailability of dietary iron in the digestive pathways are very important. Understanding the chemical properties of iron and their behaviour during intestinal transit is important for developing appropriate strategies for prevention and management of anaemia.

India is battling with anaemia

Notwithstanding the abundance of iron in Nature, iron deficiency anaemia is the most common public health problem in India today. The prevalence of anaemia is significantly higher among children and women of child-bearing age National Family Health Surveys (NFHS) 2, 3, 4, and 5[1-4] reported that prevalence of anaemia in preschool children was 74, 79, 58 to 67% respectively; Nair etal[5] reported that 40.5% of rural children were anaemic. Comprehensive National Nutrition Survey (CNNS) reported a lower prevalence from Hb estimation done from intravenous blood sample[6]. NFHS 2, 3, 4 and 5[1-4] reported that the prevalence of anaemia in reproductive age women was 52, 56, 53 to 57%, respectively.

Anaemia Mukt Bharat envisages steep reduction in prevalence of anaemia in all age physiological groups (Figure 1). Considering the changes in prevalence of anaemia between NFHS 4 and 5, it would be difficult to achieve the target set for reduction anaemia in POSHAN Abhiyaan. It is imperative to understand the factors responsible for the high prevalence of anaemia and devise effective dietary and holistic interventions to accelerate the pace of the reduction in anaemia and achieve the targets set in Anaemia Mukt Bharat. A closer look at some of the dynamic changes in dietary iron absorption and bioavailability and basic mechanistic principles involved in iron absorption may provide simple dietary methods of addressing iron deficiency anaemia.

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Fig 1 Vision: Anaemia Mukt Bharat

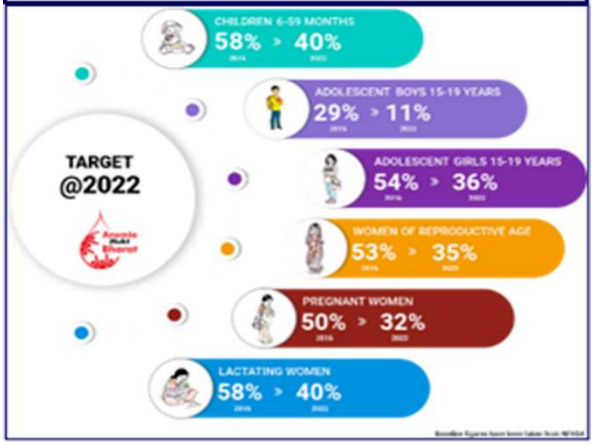


Table 1 Iron absorption (%) from habitual Indian diets in different physiological groups

Physiological Group	Method	Type of Diet	Bioavailability %
Men[9]	Radio-isotopic method	Ragi	1.6
		Wheat	2.2
		Rice	3.6
Iron deficient women[10]	Stable isotopic studies	Millet	4.6
		Wheat	8.3
		Rice	11.2
Boys 13-16 years[11]		Rice	8.6
Girls 13-16 years[11]		Rice	9.7

Dietary iron absorption and bioavailability from Indian habitual diets

Humans derive iron from their habitual daily diet. Iron is found in food as either heme (blood or muscle tissue from animals) or non-heme iron (from plants). In India, the major part of dietary source of iron is from plant foods (90-95%) and the rest is derived from foods of animal origin Diet surveys have shown that rice, wheat, and millets are the widely consumed staples in different regions of India and they provide about 60% of total dietary non-heme iron. Over time diet surveys have shown that there is an upward trend in the iron density of Indian diets[8].The contributions of iron from other food groups are 15% from pulses, 12% from vegetables, 6.5% from fruits and 4% from flesh foods[7]. The overall contribution of iron per 1000 kcal is about 8.5 mg across the states in India. There are regional differences in intake of cereals, rice as the staple have lowest (7 mg iron/1000 Kcal) and wheat and millets together as the staple have the highest (12 mg iron/1000 Kcal) iron content/1000 kcal.

Heme iron is well absorbed whereas bulk of non-heme iron is relatively poorly absorbed. Time trends in rates of iron absorption among various segments of the population in India are shown in Table 1.

These studies have had provided the basis for deriving dietary intake recommendations of iron for Indian by the ICMR Expert groups in the year 1989[12], 2010[13] and 2020[14] (Table 2). Improvement in technology for assessing iron bioavailability, has made it possible to generate data on iron absorption values among vulnerable segments of the population like women of reproductive age and children[9,10,11].

The Indian Food Composition Tables had also has undergone updating and revision[15]. However, estimated iron content of Indian diet has remained between 7-9 mg/1000 kcal. A major emphasis in the last two revisions of recommended dietary intake of

Fig 2 Determinants of iron absorption

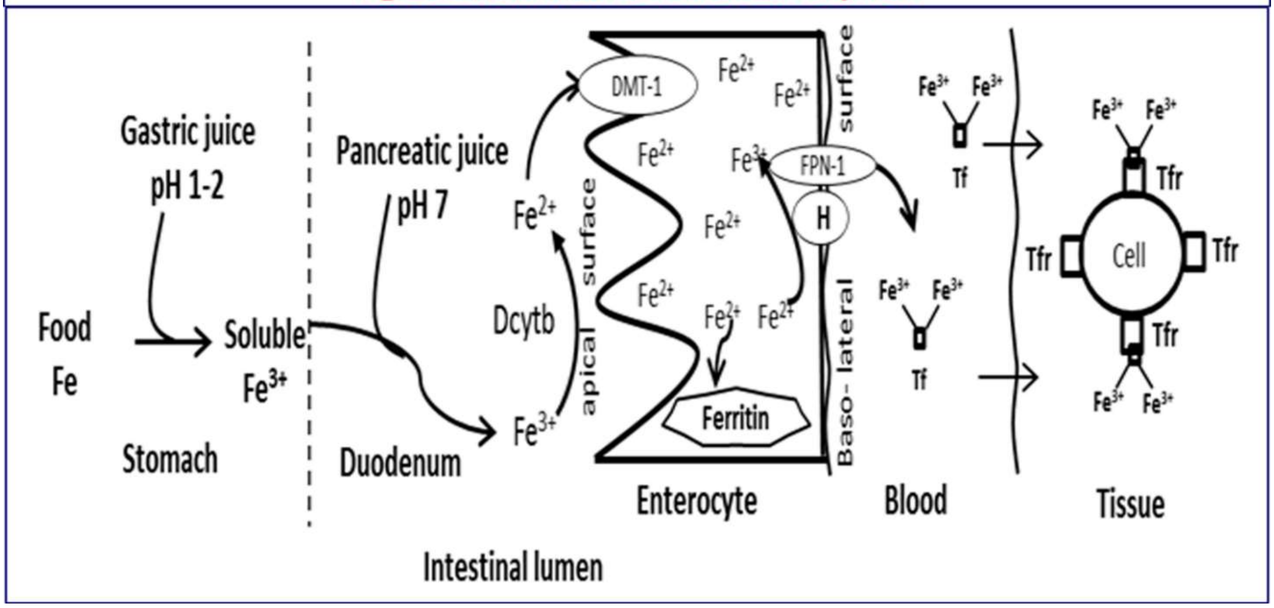


Table 2 Iron requirements (mg/day) for Indians					
Group	1989	2010	2020		
			EAR	RDA	TUL
Men	28	17	11	19	45
Women (NPNL)	30	21	15	29	45
Preg Women	38	35	21	27	45
Lact Women	30	21	21	23	45
1-3 yrs	12	9	6	8	40
4-6 yrs	18	13	8	11	40
7-9 yrs	26	16	10	15	40
Boys 10-12 yrs	34	21	12	16	40
Girls 10-12 yrs	19	27	16	28	40
Boys 13-15 yrs	41	32	15	22	45
Girls 13-15 yrs	28	27	17	30	45
Boys 16-18 yrs	50	28	18	26	45
Girls 16-18 yrs	30	26	18	32	45

1989 - Dietary iron absorption: Adult men, children and adolescent boys -3%, adult women, lactating women and adolescent girls -5% and pregnant women -8%
2010 - Adult men, children and adolescent boys and girls -5% and adult women, lactating women pregnant women -8%
2020 - Children 1-3 y, 4-6 y and 7-9 y- 6%, Adult men and women (NPNL), lactating women, boys and girls 10-12 y, 13-15 y and 16-18 y -8% and pregnant women -12%.

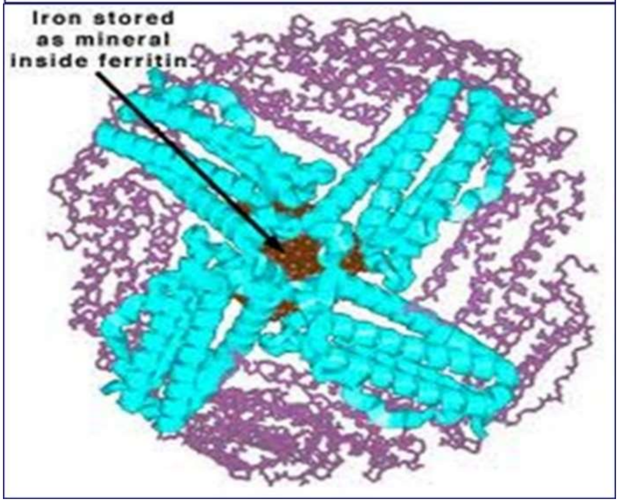
iron for Indians was an advisory with respect to last two revisions of recommended dietary intake of iron for Indians was an advisory with respect to intake of the co-nutrient vitamin C (ascorbic acid) of at least 20 mg/1000 kcal for acquiring and meeting the iron requirements in Indians. Clearly the above observations have focused scientists in addressing iron bio-availability as the core strategy to achieve significant reduction in the prevalence of iron deficiency anaemia in our population. A detailed explanation of the iron solubilization and interactions with dietary ligands that impede and enhance iron absorption during the gastrointestinal transit of iron is provided in the following paragraphs.

Food iron solubility and interactions with dietary ligands

Gastric pH and solubility: Solubility of dietary iron is greatest at acid pH 1-2 of the stomach. At this

Table 3a Food sources of iron[15]		
Food Group	Sources of non-heme iron	mg/100g
Cereals	Rice (milled) Whole wheat atta	0.7 mg 4.1 mg
Millets	Bajra, ragi, jowar,	6-4 mg
Pulses and legumes	Lentil, Bengal gram dal, Rajmah, cow pea, Black gram dal, green gram and red gram dal, green pea	7-4 mg
Green leafy vegetables and other vegetables	Gogu green and red, Fenugreek, amaranth green and red, parsley, drumstick leaves, ponnanganni, spinach, cluster beans	9-3 mg
Poultry and animal	Liver of pork, beef, chicken, and sheep	21-7 mg

Fig 3 Structure of phytoferritin



pH, majority of dietary non-heme iron which is in the ferric form is soluble but ferric iron becomes inaccessible at higher pH (>6) of duodenum. It is well known that food matrix constituents such as phytates, polyphenols (inhibitors) and organic acids (promoters) like vitamin C can influence the solubility of iron in the gastric milieu and render it more accessible or inaccessible for absorption at the duodenum (Fig 2). Determinants of iron absorption process include gastric solubility, duodenal transit and interaction at the apical absorptive surface with transporter DMT-1and internalization. Post internalization iron has two pathways: one directed towards iron exporter ferroportin (FPN1) at the basolateral surface and the second, the excess stored in mucosal ferritin. Systemic transfer and cellular uptake occur through transferrin-transferrin receptor mediated processes (Fig 2).

Dietary sources of iron

Bio-availability of dietary micronutrients is defined as the proportion of nutrient that is actually available for absorption and utilization by the body. In the case of

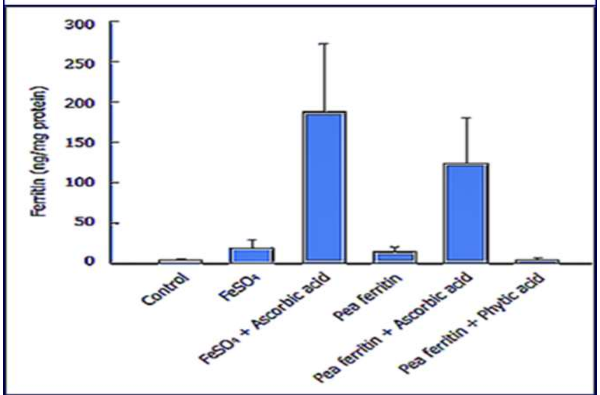
iron, absorption is synonymous with bioavailability, as there is no regulated excretion of iron. Variations

Table 3b Food sources of vitamin C and polyphenols		
Fruits and vegetables	Vitamin C (mg/100g)	Total Polyphenol (mg/100g)
Guava (pink flesh)	222	321
Guava (white flesh)	214	97
Mango (green)	90	580
Strawberry	50	135
Lemon Juice	48	24
Sweet Lime Pulp	47	117
Ripe Papaya	43	18
Parsley	133	47
Capsicum (yellow)	127	8
Capsicum (green)	123	11
Cabbage (violet)	44	60

Table 4 Food stuffs and nutrient composition of regular and modified meals[11]

	Girls		Boys	
MENUS	Regular meal	Regular meal + guava	Regular meal	Regular meal + guava
Rice (g)	450	450	400	400
Potato-Tomato *Curry* (g)	100	100	100	100
Sambar (g)	100	100	150	150
Butter milk/Yogurt (g)	100	100	200	200
Guava (g)		100		100
NUTRIENTS				
Protein (mg/meal)	10.2	9.6	9.8	9.8
Iron (mg/meal)	10.8	13.3	11.4	10
Zinc (mg/meal)	2.6	2.7	2.8	2.8
Ascorbic acid (mg/meal)	7.8	188	10.4	190.4
Phytate (g/meal)	0.3	0.2	0.4	0.3

Fig 4 Digestive stability of iron from pea ferritin



in bioavailability of dietary iron are determined by the dietary source of iron rather than the absolute amount of iron ingested. While the absorption of heme iron is unaffected by dietary promoters and inhibitors, the non-heme iron is subjected to the interplay of dietary factors. Plant sources of iron are present as phytoferritin in cereals and pulses and other food matrices. The iron present in phytoferritin is in a complex of ferric form (Fe^{3+}) ferrihydrite [FeO(OH)]8[FeO(H2PO4)] stored in the inner sphere of a apo-ferritin protein (Figure 3).

The digestive stability of phytoferritin

In order to develop effective dietary strategies for improving bioavailability of non-heme iron, it is important to understand the mechanism of release and intestinal uptake of iron from plant sources. For assessing the digestive stability and fate of iron from phytoferritin, we have isolated phytoferritin from dried pea (Pisum sativum) seeds and subjected it to various in vitro human gastric simulations. Structural changes were characterized using capillary electrophoresis, gel filtration and circular dichroism spectroscopy. The bioavailability of ferritin iron was assessed using coupled in vitro digestion/human enterocyte cell line Caco-2 cell model bioavailability system (Fig 4).

At gastric pH in the presence or absence of the major proteolytic enzyme, pepsin, there was a total loss of quaternary and secondary structure of pea ferritin and no evidence of existence of native form of ferritin, suggesting that iron was released into the gastric milieu. The induction of Caco-2 cell ferritin formation (surrogate marker of iron bioavailability) upon feeding of gastric digesta of pea ferritin showed induction similar to that observed with ferrous sulphate used as the control. The directional response of pea ferritin and ferrous sulphate in the presence and absence of vitamin C (ascorbic acid) and phytic acid were also similar.
These results suggest that the solubility, release and interactions with dietary ligands are similar for iron sources derived from pea and ferrous sulphate[16]. These steps are essential and critical for modulating bioavailability of dietary iron. These findings have translational value in terms achieving synergy between nutrient intake and gastric milieu in order to improve iron absorption from plant foods.

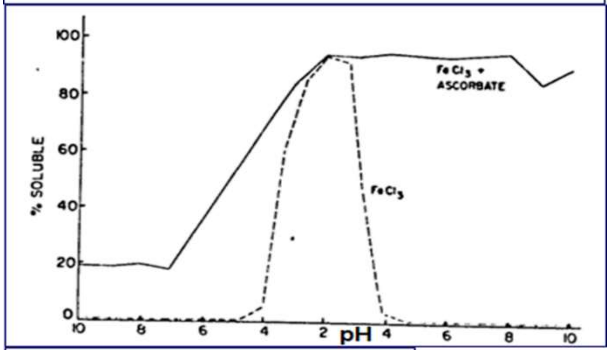
Food and nutrient synergy for improving iron bioavailability

Food synergy supports the idea of dietary variety and of selecting nutrient rich foods with non-heme iron (cereals and pulses) along with fruits rich in vitamin C (Table 3a and 3b). The scientific reasoning behind this relies on the fact that the interrelationships between constituents in foods are significant, in this case between iron and vitamin C. Further, the balance between constituents (iron and vitamin C molar proportions) within the foods and how well the constituents survive gastric digestion, the extent to which they appear biologically active

Table 5 Iron bioavailability from regular meal and with guava[11]

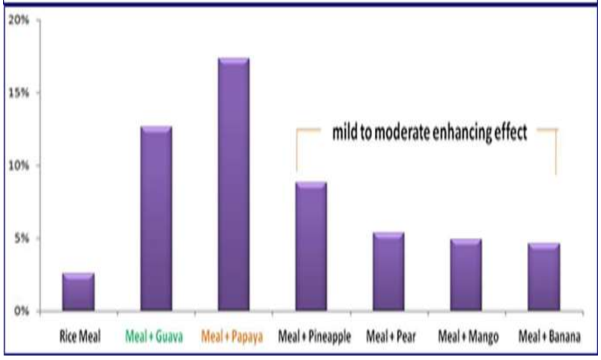
Details	Girls	Boys
	Absorption % N=15	Absorption % N=14
Regular meal	10.3 ± 6.46 (6.7 - 13.8)	11.6 ± 4.12 (5.8 - 17.4)
Regular meal+guava	23.9 ± 11.21 (17.6 - 30.1)	19.2 ± 8.38 (14.4 - 24.0)
Difference	14.2 ± 14.50 # (6.1 - 22.2)	10.6 ± 10.44 # (4.6 - 16.6)
Reference dose	58.23 ± 22.23 (46.38 - 70.08)	48.98 ± 16.00 (40.45 - 57.50)
# Paired samples t test, P=0.002		

Fig 5 Solubility of ferric iron at varying pH with and without Vit C



Broken lines without Vit C; Solid line with Vit C

Fig 6 Effect of fruits on iron absorption from a rice meal



at the cellular level decide the extent of enhancement in iron bioavailability. Observational studies show powerful link between dietary pattern and health outcomes such as Mediterranean type and NCDs. However, there are very few studies exploring dietary pattern for improving iron bioavailability from habitual Indian diets.

Dietary diversification is a sustainable approach to promote the combination of different types of foods to enhance the absorption of non-heme iron. Ferric iron solubility at pH 2 and beyond as a function of pH is a critical determinant of iron absorption (Fig 5). Solubility is retained beyond pH 2 in the presence of ascorbic acid (solid line) and reduced and become insoluble in the absence of ascorbic acid (broken line). Studies have shown that the presence of both non-heme ferric iron ($Fe3+$) and vitamin-C at an acidic pH is necessary for iron to be soluble at higher pH. Such interactions ensure reduction of ferric iron to ferrous ($Fe2+$) iron and higher solubility at the pH of duodenum. This form of iron is also readily exchangeable and interacts with the uptake transporter DMT-1 at the absorptive surface of the gastrointestinal tract (Figure 2).

The above fundamental findings paved the way for studies in improving bioavailability of iron by inclusion of vitamin-C rich foods and beverages such as lime juice, papaya, orange juice and guava to meals[17]. A two to three fold enhancement of non-heme iron absorption have been reported using single meal radio isotopic studies (Figure-6). Based on the results from the above single meal studies, we performed two studies. The study -1 was carried out to assess the extent of improvement in iron bioavailability from a regular meal consumed with a vitamin-C rich fruit, guava. The subjects were adolescent boys and girls of 13-15 yrs from residential schools in Hyderabad, Telangana and were fed their regular noon rice-based meal with or without 100 g guava and stable isotopic tracer of iron to trace the iron (Table -4). The fractional absorption of iron from meal with guava (22%), compared with regular meal (9%), was significantly ($P < 0.05$) greater in both girls and boys (Table 5). There was no significant effect of gender on iron bioavailability from meals. We conclude that simultaneous ingestion of guava fruit with a habitual rice-based meal enhances iron bioavailability more than two times in adolescents[13].

The study-2 was a RCT to examine whether regular consumption of guava as part of a meal improves iron status and reduces iron deficiency and provides functional benefit. The primary objective of the study was to assess the impact of providing 25 g guava along with a cereal/pulse-based supplementary meal on iron status and cognitive development of children 24-48 months of age. Secondary objectives included examining the

Fig 7 Changes in plasma vitamin C and folate levels in guava, banana or cucumber fed groups

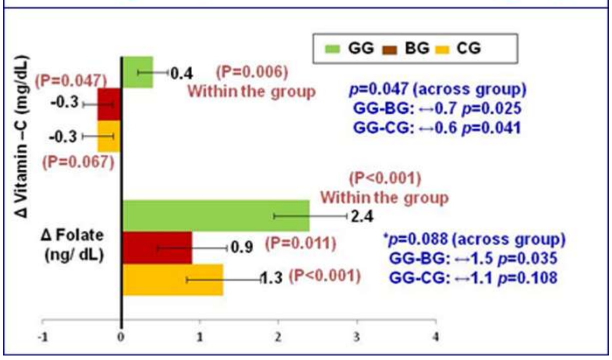
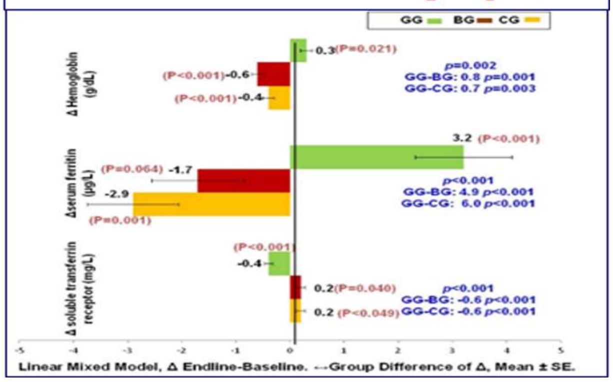


Fig 8 Changes in iron biomarkers in gauva, banana or cucumber fed groups



impact of such intervention on child growth and morbidity outcomes. We selected ICDS platform to minimize confounding factors like coverage under of health programme, socio economic background, logistics issues, community acceptability and cost effectiveness.

This study had a preparative phase involving formative research component to contextualize and establish the supply chain and this phase was followed by operationalisation of the intervention. We implemented the community suggestion by giving cucumber to the active control group and banana to the control group. This modification ensured compliance and made the trial acceptable to the community. Sixteen villages were randomized into 3 intervention groups (guava, banana and cucumber groups) with 5 villages (n=136) in the guava group; 6 villages (n=137) in the banana control and 5 villages (n=129) in the cucumber as an active control group.

Delivery, portioning and monitoring of intervention were all tested in the formative phase. The most important operational issue was identifying and engaging a proficient local fruit vendor, who was always available near the study site, ensured both feasibility and sustainability. It also helped the community to perceive themselves as important stakeholders in the trial. The cost estimate of Rs 0.80 per child per day was a realistic estimate as the charges were paid to the trader appointed for distributing fruits to all the AWCs[18].

The villages were randomly assigned to receive 25 g guava, banana or cucumber along with SNP-meal for 140days. Micronutrient biomarkers (iron status, vitamin-C, vitamin B12 and folate), cognitive development, anthropometric indicators (WAZ, HAZ, WHZ), and morbidity were assessed at baseline and endline. A linear mixed model and generalized estimating equation were applied to compare changes in outcomes measures across the groups. The findings are presented in Figures 7-9.

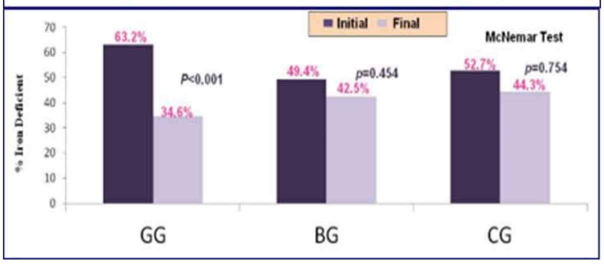
Iron to vitamin-C molar ratio in the guava group was 1:12 compared to 1:1.4 in the control groups which resulted in significantly higher plasma vitamin-C. Guava fed group had higher serum folate levels compared to banana and cucumber fed groups (Figure 7). In the guava group haemoglobin and serum ferritin were higher and serum transferrin receptors were lower as compared to the banana and cucumber group (Figure 8). These data indicated improvement in iron biomarker status and reduction in the prevalence of iron deficiency in the guava fed compared to banana and cucumber fed groups (Figure 9).

Prevalence of acute respiratory infection (ARI) was lower in the guava group as compared to the banana and cucumber groups The intervention had no impact on cognitive development or growth. The data from the study indicate that, diversifying a cereal-pulse-based meal with guava increased meal vitamin-C content, thereby reduced iron deficiency and ARI-related morbidity[18]. The efficacy reported in this study is consistent with the impacts seen from the provision of iron supplementation (change in haemoglobin 0.5-0.7 g/dL and in serum ferritin 11.6-21.4μg/L) and iron-fortified foods (0.3-0.5 g/dL and 0.6-4.7μg/L). Thus, this approach represents a valid and scalable strategy to address iron deficiency among young children.

Efficacy trials on food and nutrient synergy

There are only a limited number of efficacy trials to measure the impact on iron status following a regular consumption of a vitamin-C rich fruit along with a habitual meal. on iron status are limited. To date, there are reports of four RCTs that tested food-based approaches to tackling iron deficiency The trial in rural Mexico did not see a positive effect on iron status among iron-deficient women fed limeade with meals for 8 months[19]. The lack of positive impact has been attributed to high amounts of inhibitors in the meals; it was suggested to utilize 1 to 12 molar ratio of iron to vitamin-C. A RCT among 6-9-year-old school children in northern Mexico tested consumption of 300 mL guava juice with wheat flour-noodles and reported a marginal effect on haemoglobin and ferritin[20]. A third trial, tested the provision of guava, papaya, and oranges with a soya-based diet among Indonesian pregnant women showed a positive effect on haemoglobin and ferritin, but only among iron-deficient pregnant women[21]. Finally, an RCT in New Zealand that tested the effect of supplying two kiwi fruits with iron-fortified breakfast-cereal for 16 weeks to women 18-44 years of age reported improvement in ferritin, but not in haemoglobin, among women 18-44 years of age having two kiwi fruits with iron-fortified breakfast-cereal for 16 weeks[22]. We looked at the intake data of iron absorption promoting foods such a fruits, vegetables and flesh foods and data on

Fig 9 Prevalence of iron deficiency (initial and final) in guava, banana or cucumber fed groups



severity of anaemia across states in India to assess the trends in prevalence of anaemia and dietary pattern[6]. Data from this analysis indicated that prevalence of anaemia was lower in states which have higher intake of these food groups.

In our trial, the iron to vitamin-C molar ratio was 1:12 with the addition of 25 g of guava fruit. This fruit appears to be an ideal choice for achieving the desired molar ratio of iron and vitamin C. Guava has the advantage of having good shelf life at ambient temperature and is locally available for about 8-9 months from July to March. It can be easily portioned to satisfy the needs of pre-schoolers to adults. It is suggested that fruit rather than in juice form should form part of the meal as it provides environment conducive for establishing food and nutrient synergy concurrently.

Thus, a holistic approach that can simultaneously address various aspects of iron nutrition is needed. A number of potential dietary sources that contain adequate amounts of vitamin C need to be urgently promoted, including minimally processed leafy and other vegetables. This is possible only if the food environment is conducive with respect to accessibility, availability, affordability, absorption by the households and accommodation by food vendors. These five dimensions of the food environment can be hindering/promoting/sustaining factors for perishable foods and their influence on diet-diversity has to be contextualized and addressed, particularly, proximity to food stores and vendors' willingness to accommodate consumer demand[23].

Summary and conclusions

Anaemia continues to be a major public health problem in India. POSHAN Abhiyaan has set time frame and targets for achieving reduction in anaemia. The major etiological factors responsible for the high levels of anaemia are lower intake of iron combined with poor bioavailability of dietary iron. National strategies are in place to improve iron intake but no effort has been made to improve bioavailability. Considering the chemical properties of iron, concurrent intake of foods rich in iron and vitamin C is essential for making a significant reduction in iron deficiency anaemia. The long-term public health strategy of dietary diversification and food-based approaches pose considerable challenges before they can be implemented on a nationwide scale. Simple dietary diversification of inclusion and consumption of vitamin C fruits such as guava with main meals can enhance iron bioavailability by more than two times. This strategy has been tested and found to impact all iron status indicators uniformly when introduced into the existing supply chain of a national nutrition sensitive food-based platform. Introduction of this can be a core strategy to realize the vision of Anaemia Mukt Bharat. The role of excess iron and its unique chemistry causing concerns for intestinal oxidative stress and gut health in general has drawn attention to the need to reduce dose of iron supplementation. In this context, the recommendations of the ICMR expert committee of 2020 to implement estimated average requirement (EAR) for public health and nutrition programmes in India has considerable merit. This EAR metrics of iron reduced the gap between the requirement and the dietary intake compared to the currently used metrics based on the 2010 recommendations of ICMR. The new recommendation also provides the tolerable upper limits (TUL) of iron intake in a day (40 mg for children and 45 mg adults) which can be applied as a guard against iron overload and its health consequences, especially on the gut health. Enabling food environment is needed to bring about a behavioural change of habitual intake of vitamin C rich fruits with main meals. Its inclusion as a core strategy in the existing nutrition sensitive interventions can yielded significant results in controlling iron deficiency anaemia.

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NUTRITION NEWS

The **54th Annual Conference of Nutrition Society of India** is scheduled to be held on 22nd and 23rd of December 2022 at the National Institute of Nutrition, Hyderabad. The theme of the Conference is “Sustainable Healthy Diets - Health for All”.
In addition to the main Conference, two pre-conference workshops will also be organized on 21st December 2022. Workshop 1: Food Labelling - Healthy Food Choices and Workshop 2: Orientation on 'Research Methodology’

FOUNDATION NEWS

The **Third Dr C Gopalan Memorial Webinar** is scheduled to be held on 11.10.2022 between 10.00 AM and 1.00 PM The theme of the Webinar is “**Action plan to mitigate health and nutrition challenges associated with climate change”.**

Dr SK Singh: “Overview of India’s Health Action Plan”
Dr D Mavalankar: “Action plan to cope with heat stress”.
Dr A. Laxmaiah: “Nutrition impact of Climate change-and options for action”.
Dr Prema Ramachandran: “Climate change and food security”.

Dr D Prabhakaran, Executive Director, Centre for Chronic Disease Control and Professor of Chronic Disease Epidemiology, Public Health Foundation of India, New Delhi will be delivering the **C Ramachandran Memorial Lecture** on 29.11.2022 between 3.00 PM and 5.00 PM.