RESEARCH HIGHLIGHTS

1. Public Health Nutrition

- In spite of India's economic surge, the malnutrition (double burden of disease) is a major public health problem in India. In pursuance of goals of National Nutrition policy, sustainable developmental goals, WHO Nutrition Frame work and India's five year plans, emphasized to establish 'Nutrition Surveillance System (NSS) in India to provide early warning signs of malnutrition among vulnerable population groups to enable us to take immediate actions to mitigate the problem of malnutrition. In order to comply with the policy recommendations, the ICMR-National Institute of Nutrition Hyderabad has established NSS in 6 select states (Kerala, Maharashtra, Madhya Pradesh, Meghalaya, Odisha and Telangana) in India on a pilot basis. The main objective of the NSS is to track the Nutrition status of vulnerable population on regular basis and also presenting the real time data by using Tabs by AWWs to enable administrators to take immediate remedial actions for prevention and control of malnutrition.

- The burden of anemia is widely prevalent among all age groups, gender, physiological groups, which is a persistent public health problem in India. In spite of initiation of several nutrition supplementation programs like iron and folic acid in India, the prevalence anemia was not declined since several decades. Thus, it is time to look beyond iron and folic acid supplementation, and may consider for B12 deficiency. Therefore NIN has taken up B12 deficiency mapping in India and we would like to estimate exclusive contribution of B12 deficiency in total burden of anaemia. The findings of the study may enable us to recommend B12 vitamin supplementation.

- The high prevalence of stunting and low birth weight is also major public health problem in India. In order to prevent and control, NIN has developed a district level multi-component health and nutrition education intervention model to prevent and control malnutrition among vulnerable population groups. The intervention model has been implemented in 16 high burden districts in 5 states viz., Andhra Pradesh, Gujarat, Jharkhand, Telangana and Uttar Pradesh to prevent and control stunting and LBW in these districts. The study was implemented for last three years and a significant reduction in undernutrition among children and reproductive women was observed.

- As we know that the nutritional status of women living with HIV/AIDS is sub-optimal and improvement of their nutrition is very important in order to see good prognosis among them. For this purpose, NIN has developed special full meal supplementation program and implemented the same for 1 year. A significant improvement has been observed in their body weight, BMI and CD4 count.

2. Impact evaluation of Karnataka Multi Sectoral Nutrition Pilot Project

The Government of Karnataka initiated the Comprehensive Nutrition Mission to address the underlying prevalence of undernutrition and to clip the gaps in the existing/on-going nutrition programs. The mission has been implementing Karnataka Multi-sectoral Nutrition Pilot (KMNP) project with the objective to reduce malnutrition by increasing utilization of services related to nutrition services for children <3 years, adolescent girls, pregnant women and lactating mothers in the selected two blocks on a pilot basis. An impact evaluation was carried out by collecting quantitative and qualitative data using mixed methods approach with the objective to assess the impact of the KMNP interventions on the nutritional status of under three-year children and adolescent girls. The findings show that there was a significant difference in the

intervention blocks compared to the control blocks in the nutritional status as indicated by lower stunting of children and lower anemia in adolescent girls in the Intervention group compared to the control group. There was a significant difference in the intervention blocks compared to the control blocks on awareness of nutrition, health and sanitation related issues and utilization of various government programs, which were better off in the Intervention blocks compared to the control block.

3. Development of easy to use R programming package "ICMR_NIN_STAT" for commonly used statistical tests in medical research

Commonly used software/programs in medical research include SAS, SPSS, Stata, graph pad, Epi-info and Excel, which are cost prohibitive to purchase for researchers from developing countries. R programming language or Environment has become the lingua franca of statistical software over the last two decades. The R Programming Environment is a widely used open source system for statistical analysis and statistical programming. The objective of this study was to develop easy to use R programming package for commonly used statistical, medical and nutrition diagnostic functions in medical and nutrition research. An easy to use package was developed and it includes several functions for data importing and exporting, screening of data, data cleaning, data preparation, descriptive statistics, inferential statistics and reporting and common diagnostic functions in used in medical and nutrition research.

4. Technology development and efficacy testing of extruded rice fortified with iron, folic acid and vitamin B12

Fortification of rice with micronutrients using extrusion technology is considered a sustainable strategy to prevent nutritional deficiencies in general population. The objective of the present study is to assess the retention, stability and iron bioavailability from indigenously developed triple fortified rice (iron, folic acid, vitamin B_{12}) during rinsing and different cooking methods. Further, we also assessed the acceptability of fortified rice in adult human volunteers. The retention of iron during rinsing with excess water was >95%, while folic acid and vitamin B_{12} levels were reduced by ~25%. Watertight cooking (in electric cooker or on flame) of rice had no additional effect on the nutrient levels as compared to rinsed rice, implying their stability during cooking. However, cooking with excess water followed by decanting led to 50% loss of iron and ≥75% loss of folic acid and vitamin B_{12}. The dialyzable iron and ferritin synthesis in Caco-2 cells was higher from fortified rice compared to unfortified rice. Further, triangle tests in adult human subjects revealed that there are no significant sensory differences among fortified and unfortified rice. These results suggest that the iron, folic acid and vitamin B_{12} fortified rice has high retention and stability of fortified nutrients and is acceptable for consumption in adult human volunteers.

5. Effect of zinc supplementation prior to iron on iron absorption, and iron status in deficient rats: report of *in vitro* studies

The absorption of dietary iron is influenced by numerous dietary and physiological factors. We have previously demonstrated that zinc treatment of intestinal cells increases iron absorption via induction of the apical membrane iron transporter divalent metal iron transporter-1 (DMT1). Now we have investigated the effect of zinc on iron uptake, iron transporter and iron regulatory protein (IRP 1 and 2) expression and the impact of the PI3K pathway in differentiated Caco-2 cells, an intestinal cell culture model. It is found that zinc induces DMT1 protein and mRNA expression. Zinc-induced DMT1 expression and iron absorption were inhibited by siRNA silencing of DMT1. Further, zinc treatment led to increased abundance of IRP2 protein in cell lysates and in polysomal fractions, implying its binding to target mRNAs. Zinc treatment induced Akt phosphorylation, indicating the activation of thePI3K pathway. LY294002, a specific inhibitor of PI3K inhibited zinc induced Akt phosphorylation, iron uptake, DMT1 and IRP2 expression. Further, LY294002 also decreased the basal level of DMT1 mRNA but not protein expression. siRNA silencing of IRP2 led to down regulation of both basal and zinc induced DMT1 protein expression, implying possible involvement of post-transcriptional

regulatory mechanisms. In agreement with these findings zinc treatment stabilized DMT1 mRNA levels in actinomycin-D treated cells. Based on these findings, it can be concluded that zinc-induced iron absorption involves elevation of DMT1 expression via stabilization of its mRNA, viaaPI3K/IRP2-dependent mechanism.

6. Status of micronutrients and its influence on molecular mechanisms in diabetic nephropathy

Diabetic nephropathy (DN) is the most frequent cause of end-stage renal insufficiency. The results of study demonstrate altered vitamin status in chronic kidney disease (CKD) patients (with and without diabetes). Retention of vitamins in the circulation of CKD patients was associated with molecular mechanisms of kidney dysfunction. The plasma levels of minerals were found to be low in the CKD patients. These results indicate blood/plasma levels of vitamins might not necessarily represent their adequacy/inadequacy status in DN patients because cellular nutrition depends on the normal uptake of nutrients at the cellular level followed by effective utilization at the tissue level.

7. Developed a non-invasive nanoparticle mediated delivery of triamcinolone acetonide for diabetic retinopathy

Diabetic retinopathy (DR) is the leading cause of visual impairment and blindness worldwide. Current day treatment of DR relies heavily on invasive techniques such as intravitreal injections of therapeutic agents. To date, there has been no non-invasive drug delivery system reported for DR treatment. We developed a core-shell nanoparticle-based delivery system loaded with triamcinolone acetonide and evaluated its efficacy in a DR rat model. The drug loaded nanoparticles significantly improved structural and functional aspects of retina as compared. This study demonstrates the potential of a nanoparticulate delivery system for use as a topical formulation for treating DR.

8. 4-PBA prevents diabetic muscle atrophy by modulating ER stress response and ubiquitin-proteasome system

In this study the role of ubiquitin-proteasome system (UPS) and ER stress in the brain of diabetic rats and examined the therapeutic effect of a chemical chaperone, 4-phenylbutyric acid (4-PBA) was investigated. 4-PBA intervention attenuated ER stress, alterations in ubiquitin-proteasome system (UPS), and ER-associated protein degradation (ERAD) components in diabetic rats. Importantly, neuronal apoptosis was lowered in 4-PBA-treated diabetic rats. These studies suggest that altered UPS could be one of the underlying mechanisms of neuronal apoptosis in diabetes and chemical chaperones such as 4-PBA could be potential candidates for preventing these alterations under hyperglycemic conditions.

9. Proteasome inhibitory potential of cinnamon extract in prostate cancer: *In vitro* and *in vivo* studies

Cinnamon extract and its components (*Procyanidin B2, Cinnamaldehyde, Cinnamic acid and Eugenol*) inhibited the catalytic enzymes of the 26S proteasome, decreased cell viability and led to apoptotic cell death of human prostate cancer cells. Treatment with cinnamon and its compounds also resulted in suppression of angiogenic and anti-apoptotic gene markers. Interestingly, cinnamon extract and its bioactive compounds had a minimal effect in inhibiting the proteasome or decreasing the viability in normal cells. In conclusion, the results from this project demonstrate that cinnamon and its active components act as proteasome inhibitors and anti-cancer agents.

10. Impact of vitamin D deficiency on the cardiovascular function in a rat model

Herein, whether vitamin D deficiency induces oxidative stress and fibrotic changes in the rat heart and whether the changes observed can be reversed upon rehabilitation of the vitamin D

deficient rats with control diet was assessed. Vitamin D deficiency led to increase in protein carbonyls, altered activity of antioxidant enzymes and increase in nitrate levels in the heart. These results suggest that vitamin D deficiency leads to increase in oxidative and nitrosative stress in the rat heart. Further, vitamin D deficiency appeared to lead to fibrotic changes in the heart. Supplementation of the deficient rats with vitamin D corrected all the changes.

11. Studies on Xanthophylls: Dietary sources, processing, bioavailability and biological effects

A study was carried out to screen common plant foods for xanthophylls content and studied stability during storage, processing, bio-accessibility and bioavailability in rats and humans. The study generated a database on the composition of bioactive xanthophylls in commonly consumed foods using validated HPLC techniques. The study demonstrated that domestic cooking methods decreased the xanthophyll contents as compared to the fresh samples. The retention was higher in most samples when cooked by microwave or steaming methods. The storage stability of xanthophylls in fruits studied at room temperature showed higher levels as compared to those stored in refrigerator. The invitro bioaccessibility of xanthophylls lutein and zeaxanthin in vegetables ranged from 30-59%. The invivo bioavailability studies of lutein in gerbils from cooked green leafy vegetables amaranthus and spinach was 59 and 55%, bioavailability of zeaxanthin from cooked maize was 24% and bioavailability of β-cryptoxanthin from ripe papaya was 36%.

12. Prebiotic effect of legume raffinose family oligosaccharides

Effect of commonly consumed legume prebiotic oligosaccharide on high fat induced obese mice model was studied. The gain in body weight was observed to be more in high fat fed group whereas green gram fed animal group exhibited lower body weights when compared to other legume fed groups. After 18 weeks of the study, oral glucose tolerance test (OGTT) was carried to see the effect of legume prebiotics on the amelioration of insulin resistance. The results of oral glucose tolerance test revealed that high fat fed group exhibited higher glucose levels for all the time-points i.e 0,30,60,90,120 minutes respectively. Green gram fed group showed lower glucose levels when compared to other legume fed groups.

The oligosaccharide fermentation in the caeco-colon by the bacteria can give many positive health benefits as prebiotics. The prebiotic potential of legume oligosaccharides on the control of obesity high fat induced obesity was carried out in animal model. The results of the prebiotic potential of legume oligosaccharides were also promising since there was a decrease in blood glucose level, improved lipid profile, and improved body mass composition.

The ceacum sample analysis showed that increase in the shot chain fatty acid. The ceacum content analysis for gut bacteria by conventional method showed that increase in the colonies of beneficial bacterial counts.

13. Gluten intolerance in India: Prevalence, food gluten level and intake rates and whether fermented products are remedy for celiac disease?

Determination of levels of gluten in non-gluten foods such as oat and rice as well as gluten free products to measure gluten cross contamination. Estimation of gluten contamination in flour samples collected directly from mills in different rural areas to analyze cross contamination in non gluten products.

About 90-92% of food samples labeled as gluten-free are strict gluten-free, while other samples contained gluten levels marginally above levels of codex standards. Nearly 70% of local brands, 30% of mill samples and 13% of branded samples were contaminated with gluten levels above codex standards in naturally gluten-free food samples. Natural gluten-free flour samples collected directly from local mills and unbranded products contained a considerably high amount of gluten. Over 36% and 9.8% of unclaimed and claimed gluten-free products contained gluten levels above codex standards (20mg/kg).

In unclaimed products, the category in grain flours (35.9%) and oat (85%, 11.67-1830mg/kg) as the main ingredients are highly contaminated with gluten. The consumption of oats and flours from local brands and flours obtained from common flouring mill could be a high concern in celiac patients although it is of non-gluten origin, as even trace levels of gluten are risky for celiac patients.

14. A Hospital based survey on the prevalence of food allergy was conducted with the objective to assess the prevalence of reported food allergy at hospitals, in and around Hyderabad and to list the food items causing allergy, as reported by subjects which was confirmed by using skin prick test (SPT), serum IgE, serum histamine and food specific IgE estimates. The results of the study showed that almost 17% of the patients were atopic and were most sensitive to orange, papaya, guava, drumstick, prawns etc,

15. *Staphylococci* contamination and the risk associated with production of toxin in milk products

The study was planned with the objective to isolate and identify Staphylococcus from milk products and to evaluate the percentage of enterotoxin producing coagulase negative and positive strains of Staphylococcus in milk products. The results of the study showed that among 400 milk products analyzed S. aureus contamination was found to be highest in Khoa (66%) when compared to other milk products.

16. Studies on the food system of the Meitei community of Manipur and its nutritional implications

The study assessed the availability, accessibility and dietary utilization of foods consumed by the Meitei community of the lowland valley from ten different villages. Total of 313 foods were recorded to be consumed by the Meitei community through focus group discussion and indepth interview. Among those, 95 foods found to be indigenous thus analyzed for the nutrient composition and phytonutrient profile. Many of the indigenous leafy vegetables were found to be rich in micronutrients like B1, B5, B9, vitamin C, β-carotene, and minerals such as Fe, Mn, K, and Mg. High antioxidant activity was found in the leafy vegetables such as *Cissus adnata, Nelumbo nucefera* and fruit like *Euphoria longana.* Nutritional status of the Meitei community was studied in 1920 households by measuring basic anthropometry and dietary intakes were studied in 240 households. Low intake of milk and milk products was observed in all the age groups of the community. The community also had very low intake of vitamin A. However, both over-nutrition and under-nutrition was found in the adults of this community.

17. Dietary intake of aflatoxins from spices risk assessment

The study was undertaken to assess the extent of aflatoxin and ochratoxin contamination in various spices and spice blends and to perform risk assessment of aflatoxin exposure from spices from the available data on aflatoxin levels and dietary spice intake in the Indian context. Aflatoxins were detected in a total of 61/80 samples at levels ranging from 2.0-37μg/kg. The number of contaminated samples was more in chilli powder, nutmeg, spiced tea mixes, and RTE spice mix samples. All the chilli powder, nutmeg and spiced tea mixes analysed showed presence of aflatoxins. Levels exceeded the FSSAI specified maximum limits of 30 μg/kg in one chilli powder and 2 spiced tea mix samples. The highest mean and maximum aflatoxin level detected was in spiced tea mix samples followed by chilli powder samples. Co-occurrence of aflatoxins with ochratoxin A was detected in 56% of the samples. The level of aflatoxin intake calculated from mean and maximum aflatoxin levels was maximum from chilies followed by cumin powder, black pepper, nutmeg and mace.

18. Assessment of mycotoxin contamination in processed foods containing maize and groundnut

The study was undertaken to assess occurrence of aflatoxins, fumonisins, and ochratoxins in selected processed foods consumed as snacks and dietary accompaniments and based on

maize and groundnut and other cereal products. The aflatoxin exposure from ready to eat (RTE) groundnut snacks was calculated from the aflatoxin levels present in different RTE groundnut products and amount of the product consumed. The study indicated that aflatoxin levels were highest in fried and roasted groundnut snacks particularly when they contained discoloured kernels. Around 60% of these samples contained discoloured kernels which were found to contain aflatoxin levels ranging from 0.13-357µg of aflatoxin that was translated to 0.01-2.8 µg/g sample that exceeded the FSSAI limits of 10 µg/kg. Majority (78-100%) of the aflatoxin levels in these samples were contributed by discoloured kernels. From a total of 103 groundnut snack products, aflatoxins were detected in 51% of the samples with levels ranging from 1.0-660.0 µg/kg. Around 14% of the samples in fried groundnut, chikki, groundnut chocolate bars, groundnut masala powders and peanut butter had aflatoxin levels that exceeded the FSSAI limit of 10 µg/kg in RTE groundnut products. Highest aflatoxin level was observed in chikki and groundnut masala powders.

19. Investigation of mycotoxin contamination in herbal and medicinal plants and products to formulate prevention and control strategies

The present study was undertaken to investigate fungal and mycotoxin contamination in selected herbal and medicinal plants that are being utilized for health or therapeutic benefits. A total of 35 samples comprising 15 types of botanicals that are routinely used for health or medicinal benefits and included in the list provided by FSSAI and 51 powdered herbal mixes were analysed for aflatoxins, ochratoxin A and fumonisin B1. The results of analysis showed that majority contained aflatoxins (85%), 18.8% contained ochratoxin A and none showed presence of fumonisins. High aflatoxin levels that exceeded 20 µg/kg were detected in Daru haldi, Brahmi, Bhoj patra, Amla seeds, and dry ginger. The study carried out indicated the potential for many botanicals that are being consumed for therapeutic or health benefits to get contaminated with mycotoxins through the use of contaminated raw materials in their preparation.

20. Assessment of chemical contaminants in fresh/ packaged/ bottled tender coconut water

Samples of fresh tender coconut water (FTW) collected from Andhra Pradesh and Kerala (n=127) did not show pesticide residues. While, samples (FTW) collected from Tamilnadu (n=34) were detected with Monocrotophos (n=4) (TxD, Malayan Dwarf, COD) and Malathion (n=5) (COD and TxD) residues. Similarly, packaged tender coconut water (PTW) samples (n=126) also contained Monocrotophos (n=1) (Real active) and Malathion (n=4) (Cocojal, Madhura Coco Fresh, Cocoma and Coconad) residues. Both FTW and PTW samples contained heavy metals except Mercury and Arsenic. Only one sample of FTW collected from Kerala contained Arsenic.

21. Assessment of children who are helping their parents in agricultural farms of their own and monitoring their health and the health of their mothers with respect to exposure to pesticides

The community based cross-sectional study among the farm women (24-45 years, exposed=129, control = 134) (belonging to Ranga Reddy district of Hyderabad, Telangana), were detected with residues of OPs (Chlorpyrifos, Diazinon, Malathion and Monocrotophos) in their blood samples. This might have led to alterations in the Acetylcholinesterase (AChE) levels, oxidative stress parameters, CD cell markers, hormone levels and low levels of micronutrients viz., vitamins A, D, E, Calcium, Copper, Magnesium, Manganese and Zinc. Similar observations were made among the farm children (9-12 years: exposed = 66, control = 69 and 13-15 years: exposed = 63, control=65). However, supplementation with micronutrients (9-12 years: n = 54 and 13-15 years: n = 56) has improved the levels of vitamin E, copper, magnesium, zinc (both age groups), manganese (13-15 years) and enzymatic activities like AChE, lipid peroxidation (both age groups), catalase levels (13 - 15 years) among the post-

supplemented children. This could be due to enhanced metabolism/excretion of residues in the body.

22. Development and validation of a comprehensive index for assessing food safety at household level

A cross-sectional study conducted among primary home food preparers (N=400) in rural and urban (@200 each) areas of Telangana helped develop and validate a household food safety index (HFSI). An 87-item comprehensive index questionnaire covering variables like knowledge, practices and enabling-environment was developed and associated with presence of high risk food borne pathogens in samples. Of them, 11 index variables were found to be significantly associated with food contamination. These 11 key variables were used to develop a household food safety index (HFSI) that can rapidly ascertain food safety status at household level. These 11 parameters were collapsed into five context-specific 5 key messages. A communication campaign was carried out among households (N= 120) using the 5-keys to food safety and after the campaign, significant improvement was observed in HFSI scores.