

# RESEARCH HIGHLIGHTS

In its relentless efforts, the National Institute of Nutrition (NIN) continued its research in different thrust areas pertaining to human nutrition and health. As the life expectancy is increasing the prevalence of degenerative diseases is also on the rise. Keeping this in view, National Nutrition Monitoring Bureau (NNMB) carried out surveys on the prevalence of degenerative diseases and the dietary intake of the population in different states of the country. During this year under the Country Investment Plan, technology transfer for the development of micronutrient-fortified foods to the State Government enterprise was given priority. In addition, technical assistance was also provided to the AP State Civil supplies department to fortify and supply wheat flour to the community through Public Distribution System. Emphasis was given to other priority areas like tribal nutrition, beneficial effects of antioxidants in human health and also as stabilizing agents in edible vegetable oils. In addition, there is a paradigm shift in the nutrition research related to degenerative diseases. Fostering this vision, NIN has taken up new approaches in identifying molecular basis of degenerative diseases such as link of  $\alpha$ -Crystallin between diabetes and cataract and role of resistin in diabetes. A series of studies on dietary fats, micronutrients, women's health, food safety and health benefits of ginger were also carried out. The role of NSS volunteers as change agents in nutrition education in the community was studied. Here are the highlights of the research carried out during the year:

## 1. COMMUNITY STUDIES

### 1.1 Health and nutrition status of Tsunami affected population living in the relief camps in Andaman & Nicobar Islands

A rapid survey was carried out during the months of April/May 2005, to assess the health and nutritional status of Tsunami affected population living in the relief camps. In addition, a survey was carried out in the two hostels (one each for boys and girls) in students studying in 10<sup>th</sup> and 12<sup>th</sup> standards

in the affected islands, to assess their nutritional status. A total of 2513 individuals from 28 relief camps established in nine Islands for Tsunami affected population in Andaman and Nicobar Islands were covered in the survey.

The levels of consumption of various food groups observed in the current survey were, however, better than those reported for their rural counterparts of mainland except other vegetables, milk & milk products, the intake of which was low. The median intake of various nutrients (per CU/day) by the households barring proteins was less than the RDA. The data revealed that the extent of undernutrition among preschool children in the relief camps of Andaman and Nicobar Islands was significantly lower than that reported for their rural counter parts of mainland. The girls were nutritionally at a disadvantage as compared to boys among both the Settlers and Nicobarese. The adult Nicobarese were better in their nutritional status as compared to the settlers.

### 1.2 Prevalence of Vitamin A deficiency (VAD) among preschool children of rural India

As a part of the survey on "Prevalence of micronutrient deficiencies" Vitamin A deficiency (VAD) was investigated among the vulnerable groups of rural population in the States of Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu and West Bengal. Out of the total of 71,591 preschool children from 633 villages 3,934 samples were analyzed for blood Vitamin A levels using Dry Blood Spot (DBS) technique to assess the sub-clinical Vitamin A deficiency.

The result suggest that about 62% (CI: 60.3, 63.3) of children in general, had blood vitamin A levels of <20 g/dL, indicative of high prevalence of sub-clinical vitamin A deficiency. The proportion ranged from a high of about 88% in Madhya Pradesh, through 79% in Kerala to about 50-60% in the remaining States. The prevalence of sub-clinical VAD (<20 g/dL) was significantly ( $p < 0.05$ ) higher among 3-5 year children (63.1%, CI: 61.2, 65.0)

compared to 1-3 year children (59.6%, CI: 57.1, 62.1), with no significant gender differentials (Boys: 60.8%; Girls: 62.8%).

The prevalence was relatively higher (66.7%) among children who did not receive massive dose of vitamin A during the past 12 months, compared to those who received either one (61.6%) or two doses (56.3%). In the State of Kerala, where the prevalence of sub-clinical VAD was highest (79.4%), the proportion of children with dietary intake of vitamin A in amounts of <50% RDA was also high (91.8%) and that of coverage for massive dose of vitamin A was least (38.5%), compared to the remaining states surveyed.

The study revealed that the magnitude of prevalence of sub-clinical vitamin A deficiency was quite high in all the States including those states where the prevalence of clinical deficiency signs were either absent or very low. Therefore, there is a need to strengthen all the components of the National Programme for Prevention of Nutritional Blindness in the country.

### 1.3 Assessment of diet and nutritional status of individuals and prevalence of hypertension in adults & anaemia among adult men and NPNL women in rural communities

In view of increasing problems of diet related chronic diseases such as obesity, hypertension, etc a survey was carried out to assess the prevalence of obesity, hypertension among adult men and women ( $\geq 20$  years). The prevalence of diabetes mellitus (DM)/ hyperglycemia was assessed in the State of Andhra Pradesh on a pilot basis. Estimation of haemoglobin was also carried out among adult men and NPNL women. A 24hr. dietary recall survey was also carried out to assess the food and nutrient intake of all the individuals, in every alternate HH (covered for socio-demographic information).

#### Obesity

The overall prevalence of abdominal obesity in terms of waist circumference ( $\geq 102$  cms) was about 1% among men, while it was 7% among women ( $\geq 88$  cms). The prevalence of abdominal obesity in terms of waist hip ratio (WHR) was 25% and ranged from as low as 10.1% in Madhya Pradesh to a high of 40.5% in the State of Kerala among men. It was 69%

in women and ranged from a low of 36.7% in the State of Maharashtra to a high 91.8% in Kerala.

#### Hypertension

The overall prevalence of hypertension (SBP  $\geq 140$  and/or DBP  $\geq 90$ mm of Hg) was about 25%. No gender differentials were observed in the prevalence of hypertension. The prevalence tended to increase with age, from 13.6% in 20-30 years group to 56.4% in  $\geq 80$  years age group. About 60-67% of the adults were aware of hypertension, while 2-3% were currently on treatment. The prevalence of hypertension was high among the adults, who were suffering from overweight/obesity (48%) compared to normals (26%).

#### Diabetes mellitus

Estimation of fasting blood glucose among adults was carried out only in the State of Andhra Pradesh. The prevalence of diabetes mellitus (FBS levels of  $\geq 126$  mg%) was about 4% among adult men, and about 3% among women. The proportion of impaired fasting glucose FBS levels of 110 to 126 mg% was about 2% each among men and women. About 48-56% were aware of diabetes mellitus, and about 2% were currently on treatment.

About one third of adult men were currently smoking, out of them three fourths were smoking since more than 10 years. The prevalence of diabetes mellitus was high among adult men and women who were overweight/obese (9%) compared to normals (2-4%).

### 1.4 Nutritional status of tribal population in ITDA project of Bhadrachalam in Khammam District, Andhra Pradesh

A special survey was carried out to assess the health and nutritional status of the tribal population in the project area. The district has a total tribal population of about 4.5 lakhs, mainly constituted by Koyas, Lambadas/Sugalis, and Kondareddis. The study revealed high rate of adult illiteracy, poor economic status, dependency on agriculture and allied occupations for livelihood, low intake of protective foods and gross inadequacy in the intake of micronutrients. The overall prevalence of undernutrition, though low compared to their tribal counterparts of the State, it was however, higher than

their rural counterparts. The prevalence of morbidities such as fever and diarrhoea was also relatively higher compared to that reported in rural areas, indicating the problem of poor environmental sanitation and personal hygiene. The coverage of beneficiaries for immunization and supplementation of massive dose vitamin A and IFA tablets was relatively good. Poverty and poor health seeking behaviour probably contributed to aggravate the situation.

### 1.5 Acceptability of micronutrient fortified millet based biscuits-A study among primary school children

Undernutrition continues to be a major public health problem in India, affecting a large section of the communities, the most vulnerable being young children and women of reproductive age groups. In addition to the existing mid day meal (MDM) programme, the Commissioner of Civil Supplies, Government of Andhra Pradesh proposed to supplement micronutrient fortified millet based biscuits as snacks to the primary school children. The biscuits were fortified with ragi/maize/jowar in levels, so as to provide 50% of the recommended allowances per day/child, by consumption of three biscuits with a total weight of 24 g. Sensory evaluation of the fortified biscuits was evaluated. All the three varieties of biscuits viz. jowar, maize and ragi based ones were found to be equally good.

With regard to the acceptability of the micronutrient-fortified biscuits among primary school children, about 90% of the children gave a score of 'good' to 'very good' for all the characteristics studied viz. appearance, colour, texture, flavour and taste with respect to all the three types of micronutrient fortified biscuits.

## 2. CLINICAL STUDIES

### 2.1 Obstetric outcome and proinflammatory cytokine response in women with genital tract infections

There has been extensive research on maternal infection and pregnancy outcomes in developed countries, relatively, little is known in the underprivileged poor communities of India, where the problem of infections is much greater. Association of IUGR and PTD with histological chorioamnionitis and local cytokine (IL8, TNFa) response was determined.

The results revealed that height and weight of mothers were associated with birth outcome, histologic chorioamnionitis was significantly associated with linear growth of babies. In addition to nutritional factors, other factors such as inflammatory response due to genital tract infections might play an equally important role in adverse birth outcome.

### 2.2. Maternal nutrition in early pregnancy affects placental development

A study was carried out to assess and compare the placental morphology by measuring the villous structure and vascular endothelial growth factor (VEGF) and placental growth factor (PLGF) expression from conception at 7-10 weeks of gestation, of low socio-economic status (LSES) and high socio-economic status (HSES) groups, in relation to their nutritional status. The study indicated the significant disparity in placental structure between the undernourished and well nourished at a comparable gestational period and is suggestive of a predominant hypoxemic placental development in these LSES women under the stress of undernutrition.

## 3. BASIC STUDIES

### A. Micronutrients

#### 3.1 External validation of the National Facility for Dried Blood Spot Technology for Vitamin A Estimation

The National facility for Dried Blood Spot (DBS) Technology for vitamin A estimation established at the Institute is operational since March 2004.

There was a good agreement between the DBS and plasma retinol analyzed at the facility at various time points and that analyzed at the Croft Technologies after a period of one year. Thus the performance of NIN DBS facility has been externally validated.

#### 3.2 Iron and zinc bioavailability of representative Indian and US diets : Regional distribution and availability of iron and zinc from representative Indian diets

One of the main causes of iron deficiency anaemia is low dietary bioavailability of iron. It is, generally, accepted that iron and zinc deficiencies

frequently occur together because the dietary factors that impair iron absorption also affect zinc absorption. There are no RDAs for zinc in India. Therefore, it is important to obtain regional data on dietary intake and food composition and to measure iron and zinc absorption from several days of dietary consumption as meals.

There are regional, rural and urban differences in iron and zinc density and their in vitro availability, which are mainly due to the composition of major staple and phytate content in the diet. Modification of diet to improve iron and zinc availability can be achieved by replacing major staple either by improving iron content and/or minimizing inhibitor phytate. Ironically good sources of minerals are also good in phytates and the intake of absorption promoters such as fish, meat and ascorbic acid is very low.

## B. Food fortification

### 3.3 Fortification of whole wheat flour (atta) with micronutrients iron, folic acid and vitamin A - Public Private Partnership

As part of the Public Private Partnership and under Country Investment Plan, the technology of fortification of whole wheat flour (atta) with micronutrients developed by NIN has been translated to fortify and supply wheat flour through fair price shop in the state of Andhra Pradesh on a pilot scale by the AP State Civil Supplies Department, Government of Andhra Pradesh. The fortified atta branded 'VIJAYA ENRICHED ATTA' provides iron - 60 mg, folic acid - 1.5 mg and vitamin A 3300 IU per kg of atta and is priced Rs.12/kg.

## C. Dietary fats

### 3.4 Effect of sesame lignans on the oxidative stability of edible vegetable oils

Sesame (*Sesamum Indicum* Linn) has long been used as a traditional health food in India for its nutritional and medicinal value. Sesame contains substantial amounts of unique components, namely sesamin and sesamol. The higher stability of sesame oil has been attributed to its inherent lignans. The effect of sesamin and sesamol in enhancing the stability of edible vegetable oils was evaluated. The increase in antioxidant potential and

Radical Scavenging Activity (RSA) of Soyabean oil (SBO) or Sunflower oil (SFO) due to addition of lignans may possibly be due to synergism among sesame lignans and non-glyceride components of SBO (soya lignans, isoflavanoids) or SFO (phytosterols).

### 3.5 Role of n-3 PUFA in foetal programming of insulin resistance in offspring: Biochemical and molecular mechanisms

Long chain polyunsaturated fatty acids (LC-PUFA) of both n-6 and n-3 series are integral components of cell membrane and are important determinants of fetal growth and development. Studies on the effects of n-3 PUFA on fetal programming of biochemical and molecular parameters associated with insulin resistance suggests that maternal intake of Trans fatty acids (TFA) (from hydrogenated vegetable oils) may increase the susceptibility to biochemical/metabolic alterations known to be associated with increase in risk of chronic diseases.

ELOVL4 is a novel member of family of human fatty acid elongases involved in long chain fatty acids and whose function is essential for photoreceptor maintenance. These observations suggest that ELOVL4 expression may be related to n-3 PUFA nutritional status. Further, the decrease in retinal ELOVL4 expression associated with abnormality in retinal morphology in TFA fed groups suggests that TFA may affect retinal function and metabolism of long chain PUFA.

## D. Antioxidants

### 3.6 Health beneficial effects of fruits and vegetables: Total phenolic content and antioxidant activity of dry fruits

Phenolic compounds present in fruits and vegetables are reported to have multiple biological effects including antioxidant activity (AOA). The phenolic content and antioxidant activity of some commonly consumed plant foods and some preliminary data on the antioxidant activity of a few fresh fruits as natural sources of antioxidants has been generated. Dry fruits are rich in antioxidant activity and phenolic compounds appear to be significant contributors to their antioxidant activity. Consumption of dry fruits may therefore augment the antioxidant status and protect against chronic diseases.

### 3.7 Development of antioxidant rich recipes utilizing legumes as the base

Phenolic compounds are the potent ubiquitous antioxidant substances present in plant foods. An attempt was made to generate the data base on the antioxidant activity (AOA) and phenolic content (PC) of plant foods commonly consumed by the Indian population and assess the effects of different types of domestic processing on these parameters. It also involves formulating AOA rich recipes based on the data generated and assessing the effect of the consumption of these recipes on AO status in human volunteers. The AOA of salad prepared with green gram sprouts with lemon, salt and pepper was the highest among the different salad recipes tested and the one prepared from Bengal gram sprouts was the next best.

#### E. Degenerative diseases

### 3.8 Diabetic cataract and chaperone function of -crystallin

Prolonged diabetes, without proper management, can lead to various short-term and long-term secondary complications, including diabetic cataract. Accumulation of modified proteins due to unfolding and aggregation is the major molecular event in cataractogenesis. Chaperone-like function of one of the lens proteins, -crystallin, is believed to be vital for not only to prevent protein aggregation in cataract formation but also to function as a stress mediator in many other stress conditions. Studies indicate that post translational modifications such as nonenzymatic glycation under diabetic conditions has a negative impact on the chaperone function of -crystallin in terms of protecting enzymes against inactivation. Though, expression of -crystallin is increased due to hyperglycemia induced stress in many tissues including lens, there is enhanced degradation and modification. These studies provide a link between chaperone function of -crystallin and diabetic cataract. Further studies are under way to manipulate the expression and modification *viv-a-vis* chaperone function of -crystallin by dietary agents.

### 3.9. Transcriptional analysis of resistin and identification of *cis*- and *trans* acting factors regulating resistin expression

The adipocytokine resistin, a member of a family of cysteine-rich proteins known as resistin-like molecules (RELM) is also shown to be involved in inflammatory processes. Previous studies have however highlighted that resistin impairs glucose tolerance and insulin action in mice. In addition, resistin also inhibits adipogenesis in murine 3T3-L1 cells. In order to further evaluate the role of these transcription factors in the expression of human resistin, an electrophoretic mobility shift assay was performed wherein the binding of AP-1, C/EBP and c-Rel to their respective cognate oligonucleotides was characterized.

Resistin promoter sequences containing the binding sites for C/EBP, AP-1 and c-Rel shows binding with nuclear extracts prepared from corresponding cells. These experiments clearly demonstrate that AP-1, C/EBP and c-Rel present in the nucleus bind to the resistin promoter and could thereby modulate the expression of human resistin.

## 4. EXTENSION & TRAINING DIVISION

In addition to the extension and training activities, the division has carried out research activities pertaining to nutrition education.

### 4.1 Development of communication strategies to improve nutrition and health related knowledge of NSS volunteers

A study (Phase-I) using NSS volunteers as change agents to educate the community on various aspects of nutrition was conducted. The NSS volunteers were selected from the colleges of urban and rural areas around Hyderabad.

The initial knowledge levels were significantly different among the NSS volunteers of rural and urban areas (ANOVA,  $p < 0.001$ ). Intervention through nutrition education by using suitable communication materials improved the nutrition knowledge of NSS volunteers of degree colleges. Since the NSS volunteers are involved in community education programmes, such programmes help them to gain the nutrition knowledge which may in turn help them to educate the community on health and nutrition aspects.

## 5. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

### A. Food safety

#### 5.1 Effect of magnesium compounds on mobilization of deposited fluoride in rabbits

A study was conducted to assess the possible benefits of magnesium compound administration in fluorosis and its capacity to mobilize already deposited fluoride from the bones as well as to prevent new fluoride deposition and, toxicological potential of Mg salts on various organs. Simultaneous feeding of magnesium compound (milk of magnesia) reduces fluoride absorption suggesting a beneficial effect of magnesium hydroxide ingestion on fluoride retention and toxicity. Histopathology and haematological study showed that there was no adverse effect of magnesium compound in experimental animals.

### B. Cancer and xenobiotics

#### 5.2. Antimutagenicity of heat processed ginger

Spices are important constituents in the preparation of various foods in Indian culinary practices. A study was undertaken on the antimutagenicity of fresh and dry forms of ginger under commonly practiced culinary conditions. The antimutagenic effect of ginger was not altered in the extracts of ginger subjected to normal cooking conditions.

#### 5.3. Ethnopharmacological validation of bio-dynamic compounds in traditional medicine

The results of earlier studies indicate that extracts (coded 4308,4212,3107,3223 & 5322) of plants, which are traditionally used as anti-inflammatory drugs have potential antioxidant activity as evaluated by battery of in vitro tests and ex vivo test (AR. 2002-04). The present investigation was therefore undertaken to validate its anti-inflammatory potential using standard experimental animal models.

The study results suggest that aqueous and alcoholic aqueous extracts of traditional preparations Rasna panchaka has potential anti-inflammatory activity as evident from decreased

exudate volume, reduced oxidative stress and modulate levels of TNF- $\alpha$  and IL-6. The biological plausibility as evident from the study suggest that water and water plus methanol extracts of Rasna panchaka can be considered as potential candidates in the treatment of rheumatoid arthritis.

#### 5.4 Role of nutrients in environmental toxicity

The use of heavy metals like Lead (Pb), Mercury (Hg), Cadmium (Cd), Arsenic (As) etc. has resulted in the rise of their levels in environment resulting in exposure that is toxic to human health. Since one decade, reports mostly from developed countries suggest that the heavy metals (Pb, Cd, Hg, As etc.) used in industries, induce slow progressive and most of the times, irreversible damage to the nervous, haemopoietic and renal systems in population. In addition, few reports indicate their interaction with nutrients (Fe, Zn, Cu, Mg, Ca etc.) and alteration in biochemical functions specially at sub-cellular /cellular levels. The important physiological functions of essential metal ions like Iron (Fe), Zinc (Zn), Copper (Cu), Magnesium (Mg) etc. have been well established. Among the various heavy metal toxicities reported, lead toxicity is reported from all parts of the world. The study suggested that among those screened 70% had lead level above 10 $\mu$ g/dl. The haemoglobin was inversely correlated with blood lead levels of 15 $\mu$ g/dl. The serum iron levels were found to be high with blood lead levels. The correlation between zinc, Iron and lead levels indicates the interaction of nutrients and pollutants.

## 6. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

### 6.1 PCR based DNA fingerprinting of WNIN strain and its obese mutants

Two mutant obese rat strains, WNIN/Ob and WNIN/GR-Ob were developed from the existing WNIN rat colony, which is being maintained at NCLAS in an inbred status for the past 84 years. Both the mutants are obese, but WNIN/GR-Ob has impaired glucose tolerance additionally. A study was undertaken to establish genetic identity for these two obese mutant rats. The cloned product from WNIN/GR-Ob were expressed both in mutant and parental strain and thus not unique to the mutant.

## 6.2. Establishment of baseline values of body composition and blood pressure in different species of laboratory animals maintained at NCLAS, NIN - A study in rat strains

National Centre for Laboratory Animal Sciences (NCLAS) is maintaining different species of laboratory animals for biomedical research both for in house use as well as supply to other institutions. As the center is catering to the needs of several institutions including for pre-clinical toxicology testing, it has become necessary to establish normal physiological and biochemical values in the most commonly used strains of laboratory animals. Since rat strains are the most frequently used animals, initial studies were taken up in different strains maintained at the centre viz., Wistar/NIN (WNIN), Sprague Dawely (SD), Fischer - 344N (F-344N), Wistar Kyoto (WKY), CFY and Holtzman.

The study showed that there were significant differences between strains of rats in terms of body composition, physical activity, serum clinical chemistry and blood pressure. By virtue of higher body weight for age in SD rats, their total body fat was also significantly higher than other strain of rats. However, it is WNIN male rats, which had higher percentage of body fat, higher resting time, higher plasma tryglycerides, higher heart rate when compared to other strain of rats. This was followed by Wistar Kyoto strain. The Fischer-344N rats showed the least growth rate, higher night time activity. These studies show that there were differences between strains and between genders in the same strain.

## 7. PRE-CLINICAL TOXICOLOGY

### 7.1. Safety/toxicity studies of ayurvedic formulations (a,b,c,d,e) (WHO Biennium Programme)

The traditional use of Ayurvedic formulations is one of the widely accepted therapy especially in the treatment of chronic diseases viz. arthritis, asthma, infertility, rejuvenation etc. The data on safety of the Ayurvedic formulations has become important for wider global acceptance of these products.

The coded Ayurvedic formulations developed by CCRAS, MoH & FW are reported to have potential therapeutic activity in chronic diseases and hence were taken up for pre-clinical toxicity screening as per WHO guidelines. Safety of five Ayurvedic formulations "a,b,c,d&e" by acute/sub-acute toxicity tests in mice/rats were evaluated as per the protocols suggested by sponsor.

#### 7.1.1. Acute

No mortality, morbidity, weight loss and abnormal behaviour was recorded after a single exposure of a test compound with ten times of the recommended therapeutic dose after 14 days in Swiss albino mice which were exposed to the test formulations.

#### 7.1.2. Sub-Acute

Pre-terminal deaths occurred in animals receiving (therapeutic dose) 1XTD (10%), 5XTD (30%) and 10XTD (60-70%) of formulation **a** between 14<sup>th</sup> day to 28<sup>th</sup> day, while the test compound **b, c, d** and **e** did not show any behavioral changes.

There were no pre-terminal deaths in animals receiving **b** test formulations at various dose levels. The physical and physiological activities, food intake and gain in body weights were not significantly different between groups exposed to **b** test compound and animals receiving vehicle. There were pre-terminal deaths with formulation **c** of males only (10%) in all the groups of animals receiving test formulations at various dose levels. There were no histopathological changes due to formulation **c, d** & **e** in all the major organs studied.

The Institute continued its endeavours to meet its mandate through various research projects that were initiated in IX Five Year Plan. Efforts are continued to identify newer emerging areas in different fields of nutrition research from time to time to develop need-based strategies to combat nutrient deficiency disorders in the country so as to meet the goals of the Country's National Nutrition Policy.