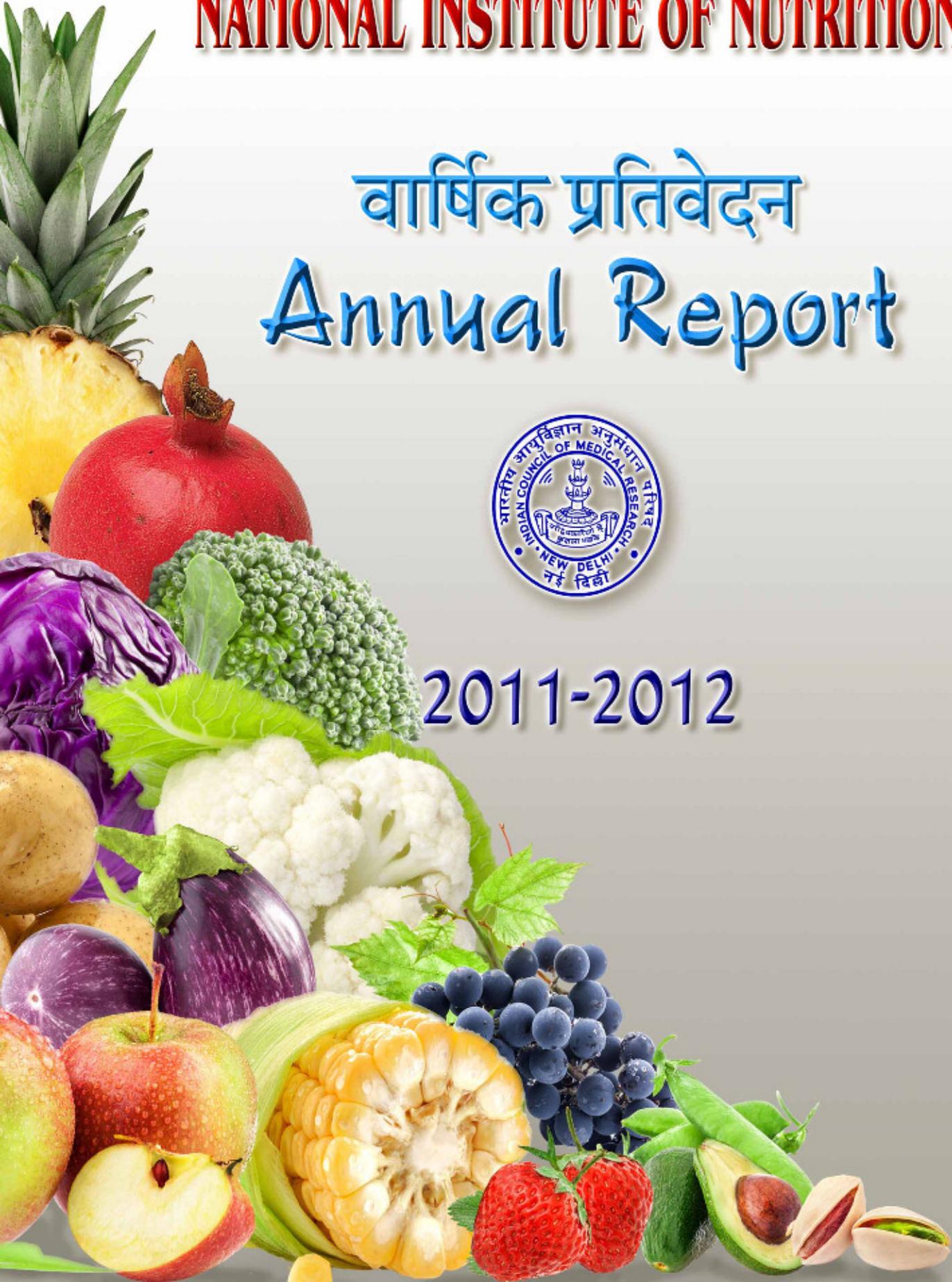


राष्ट्रीय पोषण संस्थान  
NATIONAL INSTITUTE OF NUTRITION

वार्षिक प्रतिवेदन  
Annual Report



2011-2012





# Annual Report

## 2011 – 2012





सत्यमेव जयते

## डॉ. विश्व मोहन कटोच

एम डी, एफ एन एससी, एफ ए एम एस, एफ ए एससी, एफ एन ए

**सचिव, भारत सरकार**

(स्वास्थ्य अनुसंधान विभाग)

स्वास्थ्य एवं परिवार कल्याण मंत्रालय एवं

**महानिदेशक, आई सी एम आर**

**Dr. Vishwa Mohan Katoch**

MD, FNASc, FAMS, FASc, FNA

**Secretary to the Government of India**

(Department of Health Research)

Ministry of Health & Family Welfare &

**Director-General, ICMR**



## MESSAGE



## भारतीय आयुर्विज्ञान अनुसंधान परिषद

(स्वास्थ्य अनुसंधान विभाग)

स्वास्थ्य एवं परिवार कल्याण मंत्रालय

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**Indian Council of Medical Research**

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The basic and applied research being carried out at the National Institute of Nutrition has always been of immense utility value. The thrust laid on Translational research to create direct impact on the nutritional status of the undernourished population groups and the institute's focus on ensuring food and nutrition security to people through appropriate community based search strategies have been of great help to the policy makers and administrators.

It is widely recognized that the changed scenario has given rise to new challenges and one of the is to conceptualize and define our nutrition research to address forcefully all those issues which impact the overall health and nutritional status of our people. The research endeavors need to provide able support to all the stakeholders involved in nutrition promotion. In spite of all the progress that we have made in health research, the current nutrition scenario in the country is far from satisfactory. The high prevalence of micronutrient malnutrition (hidden hunger) especially among disadvantaged communities and undernutrition among young children and women has become the main cause of concern. The emergence of chronic non-communicable diseases, especially hypertension, cardiovascular ailments and diabetes among vast segments of population groups has further complicated the situation. The nutrition transition seen in developing countries is perhaps a major reason for this occurrence and for the double burden of malnutrition prevalent in the country. Our failure to prevent the rise of chronic NCDs like diabetes indicates the need to develop region specific nutritional guidelines in the context of changed life style.

I am glad that the institute has accelerated its work in the realm of tribal health and practical strategies are being worked out to combat multiple nutritional problems affecting different tribal groups in the country. Several studies have pointed out the easy vulnerability of

most of the tribal people to malnutrition. It is hoped that institute's research expertise would pave way for the formulation of better plans and policies to augment the nutritional status of these widely scattered primitive societies.

It is heartening to note that scientific work carried out at this institute during 2011–12 in a way, reflects the scientific spirit and temper required to address several research challenges in the realm of nutrition. The studies on street children, for example, and on the other marginalized sections of people need to be more well defined, elaborate and comprehensive. Similarly, issues pertaining to adolescent health and nutrition also need a deeper insight. There is a tremendous scope for more probing studies in the fields of food safety and preclinical toxicity and let's hope that the present set of studies will lead to further serious investigations in these promising fields of research. Research in nutrition extension and education also needs to be emphasized more forcefully.

I am hopeful that more fruitful work comes out of this premier institute for the benefit of our people – both who are nutritionally vulnerable and belonging to the underprivileged sections of our societies as well as those who are becoming economically self relevant but prove to danger of over nutrition/nutritional unbalance.

I wish the Director and Staff all success in their endeavors.

  
(Vishwa Mohan Katoch)

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# RESEARCH HIGHLIGHTS

## 1. COMMUNITY STUDIES

### 1.1 Assessment of nutritional status of <5 year rural children in the State of Madhya Pradesh

Responding to a request from Madhya Pradesh Government, a study was carried out to assess i) the health and nutritional status of <5 year children, and ii) infant and young child feeding practices among <3 year children in the rural areas at the district level. Data was collected from all the 50 districts. The study revealed that about 52% of the rural children (< 5years) were underweight, 49% were stunted and 26% were wasted. These figures are lower than those reported in the National Family Health Survey (NFHS-3) in 2006. Over 78% of pregnant women underwent antenatal check-up (ANC) at least once during their pregnancy, while one third of them had three ANCs (36.4%). The proportion of newborns, who were given pre-lacteals was only 16.1% as against 58.7% reported in NFHS-3 survey. Almost all the mothers fed colostrum to their newborns. The study provided district wise information so as to enable the government to take up necessary measures.

### 1.2 Nutritional and health status of street children in Hyderabad

A study was conducted to assess the nutritional and health status of street children (n=305) of 8-17 years in Hyderabad. It was found that about 24% were smokers, 14% were consuming alcohol, 4% were smoking Ganja, 5% were inhaling whitener and 35% were consuming other tobacco products such as *ghutkha*. About 18% had dental flurosis, 2% had Bitot spots and 2% had angular stomatitis, while 16% children had skin ailments. The overall prevalence of thinness, stunting and anaemia were about 26%, 48% and 53% respectively.

### 1.3 Effect of health and nutrition education on the lifestyles and physical activities of urban adolescents

A multi-component health and nutrition education intervention was carried out to educate urban school children on healthy eating practices and physical activity. To create an enabling environment, school management and teachers as well as different educational officers were also sensitized. This resulted in a significant increase in the health and nutrition knowledge of the adolescents. In addition, there were favorable changes in their nutritional status, with lesser number of children moving from overweight to obese category and more number of children from overweight category becoming normal as compared to the control group.

## 2. CLINICAL STUDIES

### 2.1 Nutritional challenges, abdominal adiposity and type 2 diabetes in Indians

A study conducted in collaboration with London School of Hygiene and Tropical Medicine, (LSHTM), UK, aimed to examine the effect of nutritional shortage/ supplementation in early life and adulthood on the amount and distribution of body fat, and the development of type 2 diabetes and coronary diseases. The study was conducted among two cohorts from a previous study. Preliminary analysis of the data has shown that modest protein-calorie supplementation in early life was not associated with higher lean body mass (LBM).

## 3. MICROBIOLOGY AND IMMUNOLOGY

### 3.1 Molecular characterization of reshuffled Bile Salt Hydrolase (Bsh) and effect of dietary inclusion of Bsh<sup>+</sup> and Bsh<sup>-</sup> indigenous probiotic *Lactobacillus plantarum* strains of human origin on cholesterol metabolism of rats

A study was carried out on molecular characterization of reshuffled bile salt hydrolase (Bsh) and effect of dietary inclusion of Bsh<sup>+</sup> and Bsh<sup>-</sup> indigenous probiotic - *Lactobacillus plantarum* strains of human origin on cholesterol metabolism of rats. Bile salt hydrolase active (Bsh<sup>+</sup>) *L. plantarum* strain 21 reduced serum total cholesterol, LDL, VLDL and triglyceride levels in comparison to Bsh inactive *L. plantarum* strain 37. Bsh active strain 21 colonized successfully into the cecum and large intestine of the respective animal groups. Bile salt hydrolase activity helps the lactobacilli to colonize in rat gut and hence can be considered as a probiotic marker. Strains 37 and 83 exhibited negligible Bsh activity compared to 21 while no significant difference was observed among their acid and bile tolerance abilities ( $P > 0.05$ ).

### 3.2 Immune status of WNIN mutant obese rats with reference to leptin and obesity

A study that looked at the immune Status of WNIN Mutant Obese Rats with Reference to Leptin and Obesity, found that in euglycemic WNIN/Ob rat, Cell mediated immune response to Hepatitis B vaccine was impaired in obese animals. Though leptin receptor expression was intact in both the obese and lean animals, leptin signaling (JAK2 protein expression) was impaired in obese animals. In Impaired glucose tolerant WNIN/GR-Ob model too the cell mediated immune response to Hepatitis B vaccine was impaired in obese animals, whereas, the leptin receptor expression was impaired in obese animals.

### 3.3 Role of probiotics on growth and morbidity in children

A study that aimed to assess the role of probiotics on growth and morbidity in children found that after supplementation of probiotics for 9 months, there was a gradual reduction in the incidence of diarrhea in the groups supplemented with *L. paracasei* and *B. lactis* as compared to the placebo group. There was no difference in the prevalence of respiratory tract infections in all the three groups even after supplementation. There was consistent weight gain and linear growth.

## 4. BASIC STUDIES

### 4.1 Folic acid, vitamin B<sub>12</sub> status and its association with leptin and anthropometric indices of adiposity among urban adolescent boys belonging to low and middle income group, Hyderabad, India

A study was conducted to assess folic acid, vitamin B<sub>12</sub> status and its association with leptin and anthropometric indices of adiposity among urban adolescent boys belonging to low and middle income groups. Vitamin B<sub>12</sub> deficiency (39%) and folic acid deficiency (17%) were found among the adolescent boys. An age independent significant increase in body weight, body mass index (BMI) and fat free mass, waist circumference, sub-scapular skin fold thickness and decrease in HDL cholesterol were observed in the folic acid deficient group. Interestingly, vitamin B<sub>12</sub> deficient group showed a significant increase in body fat percentage and fat mass compared to sufficient group after controlling for age and economic status. It was also found that the dual deficiencies lead

to a lower HDL cholesterol levels in the adolescents. Leptin showed a positive relationship with body fat but showed no significant relationship with the two nutrients.

## **4.2 To develop a rapid and sensitive screening tool to estimate accessibility (dialyzable) of iron from food stuffs**

Measurement of iron dialyzability is used as surrogate of estimating iron bioavailability in foods. Considering that the existing conventional colorimetric method is not sensitive to detect low level of iron present in plant sources, a sensitive and rapid method of detection of dialyzable iron was developed using a combination of fluorescent probe Phen Green and a 6 well plate. This method was validated against the  $^{59}\text{Fe}$  tracer and the conventional colorimetric methods. Both the isotopic and fluorescent probe methods seem to be promising for rapid screening of dialyzable iron for selecting foods for dietary diversification.

## **4.3 Developmental origins of adiposity and insulin resistance: Role of peri/postnatal manganese status and high fat feeding in later life**

As a part of studies on developmental origins of adiposity and insulin resistance (IR), the role of peri/postnatal Manganese (Mn) status and high fat feeding in causing IR in later life were explored. Maternal Mn restriction transiently altered the body composition of male and female rat offspring. It also modulated adipocyte function and played an important role in muscle function. This study has for the first time demonstrated that maternal Mn restriction predisposes the offspring to increased central adiposity, fat deposition in liver, induction of a proinflammatory state and altered glucose tolerance specially when fed high fat diets.

## **4.4 Effect of different methods of cooking on phenolic content and antioxidant activities of pulses and legumes commonly consumed in India**

The effect of different methods of cooking on phenolic content and antioxidant activities of 11 commonly consumed pulses and legumes in India were studied. Nine out of 11 legume samples showed a maximum of 20% increase or decrease in their total phenolic content (TPC) during different types of cooking. Interestingly, during conventional and pressure cooking, whole bengal gram and *Rajmah* showed 27 and 54% increase in their TPC respectively.

## **4.5 Feasibility of using umbilical cord serum as a potential source for the growth and maintenance of pancreatic culture rat islets and assessment of their marker functions in comparison to fetal calf serum - *in vivo* and *in vitro***

A study attempted to explore the feasibility of using umbilical cord serum as a potential source for the growth and maintenance of pancreatic culture rat islets and assessment of their marker functions in comparison to fetal calf serum - *in vivo* and *in vitro*. The study suggested that hUCBS can be explored as an alternate serum supplement for FCS, making it more feasible in cell systems of human origin and can also find its application for the human transplantation programmes.

## **4.6 Pancreatic exocrine tissue as a source of progenitors/stem cells to generate insulin secreting cells**

Pancreatic exocrine tissue as a source of progenitors/stem cells to generate insulin secreting cells was examined and the methodology has been standardized for acinar cultures. Pyridoxal-phosphate (PLP) addition was protective to acinar cells and demonstrated antioxidant effects with

the addition of H<sub>2</sub>O<sub>2</sub>. PLP modulated the regulation of the transcriptional factors such as Ngn3, PDX-1, which are the master regulators for acinar lineage to beta cell formation.

#### **4.7 Exploration of basal glucocorticoid levels and their possible role in obesity and insulin resistance using WNIN/Ob and WNIN/GR-Ob rat models**

A study on exploration of basal glucocorticoid levels and their possible role in obesity and insulin resistance using WNIN/Ob and WNIN/GR-Ob rat models was completed. The results from this study suggest that 11 $\beta$ -HSD1 plays an important role in the development of obesity, dyslipidemia and insulin resistance in WNIN/Ob obese rats. Further, this study supported the hypothesis that inhibition of 11 $\beta$ -HSD1 as a key strategy to treat metabolic syndrome. This perhaps is the first study to link 11 $\beta$ -HSD1 to adipose tissue fibrosis and tissue glycogen content under obese condition. Feeding of diet rich in vitamin A decreased 11 $\beta$ -HSD1 activity in visceral fat and liver of WNIN/Ob obese rats, which is associated with decreased adiposity. Feeding of diet-rich in n-6 polyunsaturated fatty acids decreased hepatic 11 $\beta$ -HSD1 activity and increased enzyme activity in adipose tissue of WNIN/Ob lean rats.

#### **4.8 Biochemical and molecular studies on the effect of vitamin-B12 on retina under hyperglycemic conditions**

Diabetic retinopathy (DR) is one of the common complications of diabetes. Based on a hospital based case-control study, it was previously reported that vitamin-B12 deficiency could be an independent risk factor for DR. This year, an animal experiment was conducted to understand the role of vitamin-B12 in the development of DR. The results indicate a role for vitamin-B12 in retinal structure and function both in neuronal and vascular component, particularly under hyperglycemic conditions. Further, supplementation of vitamin B12 has resulted in beneficial outcomes in normalizing neuronal, vascular and inflammatory mediators under hyperglycemic conditions in the retina.

#### **4.9 Inhibition of advanced glycation end product formation on eye lens protein by rutin and ellagic acid**

Accumulation of advanced glycation endproducts (AGE) due to non-enzymatic glycation has been implicated in diabetic complications. The antiglycating potential and mechanism of action of two molecules, ellagic acid (EA) and rutin using various protein glycation systems have demonstrated. While the antiglycating action of EA seems to involve predominantly inhibition of N $\epsilon$ -(carboxyethyl) lysine (CEL) through scavenging of dicarbonyl compounds, rutin scavenges free radicals directly and also chelates the metal ions by forming complexes with them. Inhibition of glycosylated Hb formation in human blood under high glucose conditions signifies the antiglycating potential of EA. These findings establish the antiglycating potential of these flavonoids and their *in vivo* utility for controlling AGE-mediated diabetic pathologies.

#### **4.10 Amelioration of retinal degeneration in WNIN/Ob rat by vitamin A supplementation**

Earlier, retinal degeneration in a spontaneously developed novel obese rat model was reported, as WNIN/Ob rats develop retinal degeneration progressively. Studies during the current year have shown that supplementation of 26-52 mg/kg diet vitamin A alleviated the obesity-associated retinal changes in WNIN/Ob rat model, which may have implications for treatment of retinal degeneration associated with obesity.

## 5. EXTENSION & TRAINING DIVISION

### 5.1 Assessment of intra and extra individual factors on food consumption pattern in rural population – a diagnostic model approach

A project that assessed the intra and extra individual factors on food consumption pattern among rural population in Tamil Nadu using a diagnostic model approach, was carried out in two phases. In phase-I, the factors affecting food habits and food intakes in the village population were identified and education materials were developed accordingly. In phase-II, PG students from a local university were trained to educate and measure the changes among women. The study concluded that continuous and repeated exposure to nutrition communication, clubbed with interpersonal communication/group discussions can influence dietary behavior of rural women. In order to ensure sustainability of such programmes, collaboration with Social work and Women studies departments in Universities can be helpful.

### 5.2 Influence of mass media advertisements on family food purchasing patterns and efficacy of behavior change intervention

When the influence of mass media advertisements on family food purchasing patterns and efficacy of behavior change intervention, were studied, it was observed that the time spent on television viewing by women and children has an influence on their food purchasing pattern and snacking habits. A total of 1602 food advertisements appeared during the study period in television channels, popular among the study groups. Advertisements on chocolates and confectionary products were highest in number followed by health drinks and grain-based products/snacks. Advertisement of chocolates/sweets, biscuits and snacks were mostly telecast on children's channels and 63% of advertisements on health drinks were seen on other channels. Advertisements on health drinks depicted these drinks as inevitable for child's growth.

## 6. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

### 6.1 Assessment of consumption of processed and non-processed foods in India

When the consumption of processed and non-processed foods in India was assessed, it was found that the consumption of different foodstuffs and nutrients among various age groups were below the recommended levels suggested by ICMR. The consumption of processed foods was also considerably higher in both Western and Southern regions. The prevalence of undernutrition was higher among rural preschool children as compared to urban children. The prevalence of overweight, obesity and non-communicable diseases was higher among urban adults as compared to rural.

### 6.2 Effect of high fluoride and low calcium on bone metabolism in rats and genotoxicity

A study was conducted to understand the interaction of calcium and fluoride in biological system in terms of nutritional status and skeletal metabolism as well as to study the effect of rehabilitation (providing normal calcium diet and fluoride free water) on reversal of fluorosis. Nutritional status of low calcium and fluoride treated group was poor (in terms of body weight gain and body composition parameters). There was disturbance of calcium homeostasis in presence of fluoride in low as well as normal calcium treated rats. There was increased bone formation in presence of fluoride but quality of bone was poor in low calcium and fluoride group.

# research highlights

Studies on effect of reversibility indicated that the nutritional status and calcium homeostasis of rats normalized to some extent after providing normal Ca diet and fluoride free water for 3 months. There was no improvement in bone strength in animals given normal calcium diet and fluoride free water for 3 months.

## 6.3 Kidney and bone disease – Role of silica, strontium and fluoride study in guinea pigs

In a study that investigated whether silica (Si) and strontium (Sr) (with and without F) increases bone density secondary to kidney damage, it was observed that dietary intake and weight gain was reduced significantly in animals of Sr, Si + Sr, F + Sr and F+Si+Sr groups from 120 day to 180 day as compared to control. There was significantly low mineral apposition rate (MAR) and bone formation rate (BFR) in F+Sr group as compared to control. Sr and Sr+F treatment affected food intake and weight gain along with body composition and organ pathology.

## 6.4 Assessing consumer behaviour and practices related to use of food labels in India

Considering that food labelling is one of the important population-based approaches that can help consumers make healthy food choices by providing the necessary nutrition information on the pack, a consumer study was conducted in Hyderabad and Delhi to assess how many consumers among various age groups were using food labels. It was observed that only about 1/3rd of the consumers checked nutrition information and list of ingredients on labels. The reason cited for not checking the nutrient information was that the information was 'too technical to understand' and lack of nutrition knowledge. However, it was observed that women and adolescent girls who were concerned with 'fat' and 'sugar' intake were in the habit of checking the nutrition facts. A significantly greater number of consumers with higher education qualifications were checking the nutrition information. Only about 60% of the respondents checked the quality symbols.

## 6.5 Isolation, identification and characterization of pathogens from pediatric diarrheal infections in Hyderabad

Diarrheal diseases are major public health problem in children of less than five years of age. The profile of etiology of acute diarrhea changes with time, so the present study was carried out to isolate, identify and characterize enteric pathogens in pediatric population and factors associated with their occurrence.

A total of 502 stool samples were collected from children (6 months-5years of age), who were brought to Government's Children's Hospital and analysed for enteric pathogens. Seventy three (73%) percent of them were harboring one or more of the 7 major bacterial pathogens, i.e, *Escherichia coli* (36.2%), *V.cholerae* (14.5%), *V.parahaemolyticus* (0.9%), *Salmonella* spp. (18%), *Shigella* (8.3%), *Campylobacter* spp. (14.2%) and *Yersinia* (3.3%). A total of 81 stool samples were analyzed for *Rotavirus*. About 27 (33.3%) samples were positive for *Rotavirus*. Among 229 strains of *E.coli*, 61 were characterized. The Enteropathogenic *E.coli* (EPEC) accounted for 41%, Enterotoxigenic *E.coli* (ETEC) for 13.1%, *Shigella* toxigenic *E.coli* (STEC) for 34.4% and *E.coli* 0157:H7 for 27.8%.

A majority of isolates were resistant to antibiotics. More than 70% of the *E.coli* isolates were resistant to Norfloxacin, Amoxicillin, Co-Trimoxazole, Ampicillin, Ceftriaxone, Cefotaxime and Metronidazole. More than 70% of the *Salmonella* isolates were resistant to Amoxicillin, Co-Trimoxazole, Ampicillin, and Metronidazole and 65% of the isolates were resistant to Ceftriaxone,

Cefotaxime and Metronidazole. More than 50% of the *Vibrio* spp. isolates were resistant to Amoxicillin, Co-Trimoxazole, Ampicillin, and Metronidazole and 30% of the isolates were resistant to Ceftriaxone, Cefotaxime and Metronidazole.

## **6.6 Study on determination of levels of aflatoxins in stored paddy and rice of PAU 201 variety collected from 6 districts of Punjab**

In 2010 (August-September), ICMR conducted a study to assess the fungal and aflatoxin contamination of PAU-201 rice variety developed by Punjab Agricultural University, Ludhiana. About 30,000 tonnes of rice was prevented from milling and distribution through PDS, as that rice was considered to be of interior quality and contaminated.

Aflatoxins were analysed by HPLC and LC/MS/MS methods. Presence of fungus was assessed by SEM and presence of iron in discoloured rice grains was assessed by Prussian blue staining. The results of aflatoxin analysis of rice samples indicated that majority of the samples had levels <15 µg/kg and none exceeded the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011 tolerance limit of 30 µg/kg. The proportion of damaged grains exceeding the limit of 5% was observed in 85.7% of the samples. SEM and Prussian blue staining and EDX analysis of black tipped and pin point damaged rice grains did not show presence of fungal structures and presence of iron. The rice was found to be safe for consumption.

## **7. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES**

### **7.1 Estimation of body composition of laboratory animals - non-invasive and conventional methods - advantages and limitations**

Body composition analysis reveals the nutritional status and general well being of an individual. In the past, body composition of experimental animals was determined by carcass chemical method, necessitating the sacrifice of animals. But, in recent times alternative non-invasive measures like total body electrical conductivity (TOBEC) and dual X-ray absorptiometry (DXA) have emerged, which allow repeated individual measurements without sacrifice of animals. Progressive changes in the body composition of six commonly used rat strains in nutritional research VIZ., WNIN, SD, F-344N, WKY, CFY and Holtzman, were analyzed by TOBEC initially and compared with chemical method. Subsequently, animal models like Syrian hamsters, guinea pigs (NIH white and colour strains), Newzealand white rabbits were evaluated for their body composition using TOBEC and DXA and were compared with chemical method and parameters like lean body mass, fat, fat %, fat free mass were determined. It was observed that the TOBEC analysis correlated well with the carcass analysis in rats. But for hamsters, guinea pigs and rabbits DXA analysis was found to be more appropriate. In conclusion the findings equivocally shows that for body composition, analysis of lab animals like hamsters, guinea pigs and rabbits DXA is superior and constant in comparison with all methods. While for rats, TOBEC could match that carcass analysis, but it was not true for other two species. Where as in hamsters, TOBEC gave negative values, and it tends to overestimate as far as guinea pigs were concerned.

### **7.2 Localization and cloning of obesity gene in WNIN mutant rat (WNIN/Ob)**

Obesity is a multifactorial disorder affecting the significant portion of the population all over the world. The present project is concerned with the localization and cloning of the gene associated with obesity in WNIN obese mutant rats using positional cloning technique. For this, WNIN/Ob

# research highlights

mutant rats were crossed with Fisher - 344 rats and F2 generation were raised to localize the point of mutation on a specific locus using over 200 microsatellite markers. Such a genetic analysis was done both for parents and F2 progeny. Using such an approach, the gene responsible for the obesity in the WNIN rat was located on chromosome number 5. This is localized on exon genomic region of leptin receptor gene (Ob-R) which is lying on chromosome no. 5. This polymorphic region has been sequenced and the identified SNP seems to be unique and hitherto not reported). The identified sequence positioned in LepR gene was validated with known/reported SNP using bio-informatics tools and through this approach, the WNIN/Ob rat specific mutation was identified. The located coding sequence of 2679bp was found to be unique, which is a heterozygous SNP with zero degeneracy. Due to change in the A/R, the coding amino acid is changed from acidic to base. We also noticed that a specific SNP in WNIN/Ob lean LepR gene positioned in an intron G / S had changed and this also is heterozygous. The bio-informatics validation of identified SNP is completed but it is difficult to probe this in the phenotypes as it is lacking in restriction sites. Hence, the genomic region on which SNP is localized is amplified by designing primers. This is now being checked for the presence of SNP in F2 population as well as in parental strains using SSCP and sequencing.

## 8. PRECLINICAL TOXICOLOGY

### 8.1 Assessment of allergenicity potential of novel proteins expressed in genetically modified (GM) plants under varying conditions of digestion and thermal treatments

Assessment of allergenicity potential of novel proteins expressed in genetically modified (GM) plants under varying conditions of digestion and thermal treatments looked into the digestive stability to pepsin in SGF at varying pH and pepsin activity levels and heat stability. Insect bioassay of heat treated Cry1Fa1 recombinant protein showed that at a temperature of 95°C, the mortality among target insects fed was zero, indicating heat liability of the recombinant protein at this temperature. SDS-PAGE analysis showed that the band intensity of protein sample heated at 95°C was less than the untreated control at 10% conc.

### 8.2 Sub chronic toxicity study of fruit of Bt Okra containing Cry 1ac Gene in WNIN rats

Mahyco had developed Bt okra containing Cry 1Ac gene for insect tolerant trait. Its safety evaluated in WNIN rats by oral feeding for 90 days showed that there were no significant effects on body weight, serum immunoglobulins, clinical chemistry profile, hematological and histopathology due to transgenic okra.

### 8.3 Pre-clinical toxicity evaluation of recombinant GCSF-Shasun Chemicals & Drugs Ltd

GCSF (Granulocyte colony stimulating factor) was produced using recombinant DNA technology. Its safety was tested in Swiss Albino mice and New Zealand white rabbits at three dose levels namely therapeutic dose, average dose and high dose. In subchronic toxicity 5% mortality was observed in Swiss albino mice but not in rabbits. No other significant toxic effect was observed.

### 8.4 Pre-clinical safety evaluation of red gram pulses with ferric ammonium citrate

Red gram (tur dal) was fortified with ferric ammonium citrate and fed to mice/rats in intended daily dietary intake levels. Acute toxicity tests (14 days) in Swiss Albino and Sprague Dawley rats were performed and no adverse effects were observed.

# I. COMMUNITY STUDIES

## 1 Assessment of nutritional status of under five year rural children in the districts of madhya pradesh state

Despite rapid strides in agriculture and industrial growth, and consequent economic development, undernutrition continues to be a major public health problem in the developing world, including India. The most vulnerable groups are women and young children, especially in the chronically drought prone rural areas, tribal communities and urban slums.

Repeated surveys by National Nutrition Monitoring Bureau (1999) in eight states revealed that, despite very little or no change in the dietary intakes of rural population over a period of time, there was decrease in the prevalence of severe forms of undernutrition among young children with concomitant increase in normal grade. However, the proportion of children with mild to moderate undernutrition remained similar.

Recent survey carried out by NNMB (2006) in the rural areas of nine States viz. Andhra Pradesh, Gujarat, Karnataka, Kerala, Tamil Nadu, Madhya Pradesh, Maharashtra, Orissa and West Bengal revealed that, as per WHO child growth standards, among under five children, the prevalence of underweight was 40.3%, stunting was 44.6% and wasting was 19.9%. However, the figures reported by National Family Health Survey (NFHS3) during corresponding period for rural India were relatively higher (underweight: 45.6%, stunting: 50.7 & wasting: 20.7%).

In rural Madhya Pradesh, according to NNMB survey, 46.2% of under five year children were underweight, 58.7% were stunted and 24% were wasted. The prevalence figures reported by NFHS3 for Madhya Pradesh State as a whole were however much higher (underweight: 60.0%, stunting: 50% and wasting: 35%). According to NFHS3, only about 16% of the mothers in Madhya Pradesh initiated breast feeding within an hour, as against the national average of 24.5%. The study also revealed that the infant and young feeding practices in general were sub-optimal.

The objective of the study was to assess the health and nutritional status of <5 year children and infant and young child feeding practices among <3 year children in the rural areas at the district level.

The Government of Madhya Pradesh is contemplating to develop State Nutrition Policy and plan of action for implementation, in order to improve the nutritional status of the communities. Therefore, at the request of the Department of Women and Child Development, Government of Madhya Pradesh, the National Institute of Nutrition, Hyderabad had carried out surveys at district level in all the 50 districts of the state.

### OBJECTIVES

**General objective:** To assess the health and nutritional status of under 5 year children and infant and young child feeding practices among under 3 year children in the rural areas of Madhya Pradesh State.

**Specific objectives:** The specific objectives of the study were,

1. To assess the nutritional status of under 5 year children in terms of anthropometry such as heights & weights, and clinical examination for signs of nutritional deficiencies.
2. To assess the prevalence of morbidities among under 5 year children during the preceding fortnight.

3. To assess the infant & young child feeding (IYCF) practices of mothers of under 3 years children, and
4. To estimate the iodine levels in the household (HHs) salt samples used for cooking.

## METHODOLOGY

**Sampling Design:** It was a cross-sectional, community based study carried out by adopting systematic random sampling procedure.

**Sample size:** Assuming an overall prevalence of underweight (weight for age < median-2SD of NCHS reference) of 50% among <5 year children, with 5% absolute precision and 95% CI, a sample size of 383 (or say 400) children per district was arrived at.

**Selection of Villages:** A total of 20 villages were selected in each district using systematic random procedure, representing all the villages in the district based on "Population Proportional to Size" of the village.

**Selection of Households:** For this purpose, the main village and its hamlets, if any, were divided into 5 geographical areas, based on natural groups of households/streets/*mohallas*/areas etc. Households belonging to Scheduled Caste and Scheduled Tribe communities generally formed one group. From each of these groups, four consecutive HHs having at least one <5 year child were surveyed by selecting a random start, starting from the Northeast corner of the area. In the selected HH, all the children of <5 year were included in the study. In each of the selected villages, a total of 20 households (HHs) having at least one index child of <5 years were covered.

**Investigations:** The following investigations were carried out in the study;

1. Household demographic and socio-economic particulars
2. Anthropometric measurements such as heights & weights
3. Clinical examination for the presence of signs of nutritional deficiency
4. History of morbidity such as fever, acute respiratory infection, diarrhoea etc., if any, during the preceding 15 days of visit
5. Maternal particulars such as age, parity, antenatal care, TT immunization, receipt of IFA tablets, particulars of delivery and recording of birth weight
6. Infant and young child feeding practices
7. Particulars of coverage of children for all the immunizations such as BCG, DPT, Polio and measles during first year, supplementation of massive dose vitamin A among 9-59 months children and participation in the ICDS supplementary feeding programme
8. Estimation of iodine content in the cooking salt

**Data Collection:** Ten teams, each consisting of two post graduate Research Assistants (Nutritionist/Anthropologist/Social worker) and one graduate Field Investigator having proficiency in local language were recruited, trained for two weeks and standardized in various survey methodologies. Data was collected on socio-economic and demographic variables on pre-tested proforma by trained investigators. Information was also collected from mothers on feeding practices such as pre-lacteal feeding, colostrum feeding, time of initiation of breast feeding, exclusive breast feeding (EBF) and age of initiation of complementary feeding. Anthropometric measurements, such as weight (kg) and height or recumbent length (cm) were measured for all the selected children.

**Data Analysis:** The data was scrutinized and entered into the computers as soon as it was received at NIN. Descriptive statistics such as mean, standard deviations, univariate, bivariate and multi

variate analytical methods were carried out using SPSS Windows version 15.0. Appropriate statistical tools were used wherever needed.

## **Salient findings of the study:**

A total of 22,907 children (Boys: 12,387; Girls: 10,520) under 5 years of age were covered from 19,756 households in selected 1,000 villages. A total of 5,457 mothers of <12 months children, 10,320 mothers having 12-35 months children and 7,130 mothers of 36-59 months children were interviewed to assess infant and young child feeding practices..

### ***HHs socio-demographic particulars***

A majority of the HHs covered belonged to backward communities (40.5%) followed by Scheduled Tribes (29.2%) and Scheduled Caste (17.8%). About 52% of the HHs belonged to nuclear families. About half of the mothers (51.6%) were illiterate, about 41% of the HHs did not possess any agricultural land and about 51% were engaged in either agricultural or other labours. About 62% of the women were housewives. More than half of HHs (51.9%) lived in semi pucca houses and 38% in *kutch* house. Majority (72.4%) of the HHs was using bore well water, while only 9% had access to safe water. Only 12% were using sanitary latrine. Majority (96.8%) of HHs was using firewood for cooking purpose and about 75% HHs had electricity. About half of HHs (48%) were using adequately iodized salt ( $\geq 15$  ppm).

### ***Antenatal Care (ANC)***

About three fourth (78.5%) of pregnant women had undergone ANC, 36% of them had  $\geq 3$  ANCs. About half of pregnant women (49%) had registered for ANC before 16 weeks of gestation. About three fourth (78.4%) of pregnant women received IFA tablets during pregnancy, 39% received more than 90 tablets and only 20% reportedly consumed  $\geq 90$  tablets. About 79% deliveries were institutional, either in government or private hospitals. Majority (51.5%) of deliveries were conducted by a medical doctor. Birth weights were reportedly recorded in case of 69% infants, and records were available only for 43% of them. The overall prevalence of low birth weight was 19%.

### ***Infant and young child feeding practices (IYCF)***

Majority of the mothers (91.9%) fed colostrum to their newborns. Only one fourth of mothers (26%) initiated breast feeding within 1 hour and another half (48.5%) within 1-3 hours of delivery. Pre-lacteal feeds such as plain water, honey, etc., were given to 16% of the new born. Among 6-11 months children, complementary feeding was initiated at 6 months of age in about 24%, while 28% children received the same during 7-11 months of age. About 43% of children did not started complementary feeding. About 33-42% were receiving home made semisolid/solid and 44% cow/buffalo milk. About 38% were receiving complementary foods at least 3 times a day. Among 12-35 months children, about 63% children received complementary feeding in addition to breast milk, while 36% were completely weaned.

### ***Basic services to the children***

Majority of children (84.2%) were completely immunized, while about 12% were partially immunized. About 82% of 9-59 months children received at least one dose of Vitamin A during the preceding year. About 68-71% of 18-59 months children received the stipulated two doses. About 25% of 12-59 months children received IFA tablets, while only 4% of the children received  $\geq 90$  tablets and only 2% consumed  $\geq 90$  tablets.

### ***Clinical signs of nutritional deficiency***

About 98% of the infants did not exhibit the clinical signs of nutritional deficiency, while 0.4% of 0-59 months children exhibited the signs of Bitot spots indicating that vitamin A deficiency has come down over the period.

## Morbidities during preceding fortnight

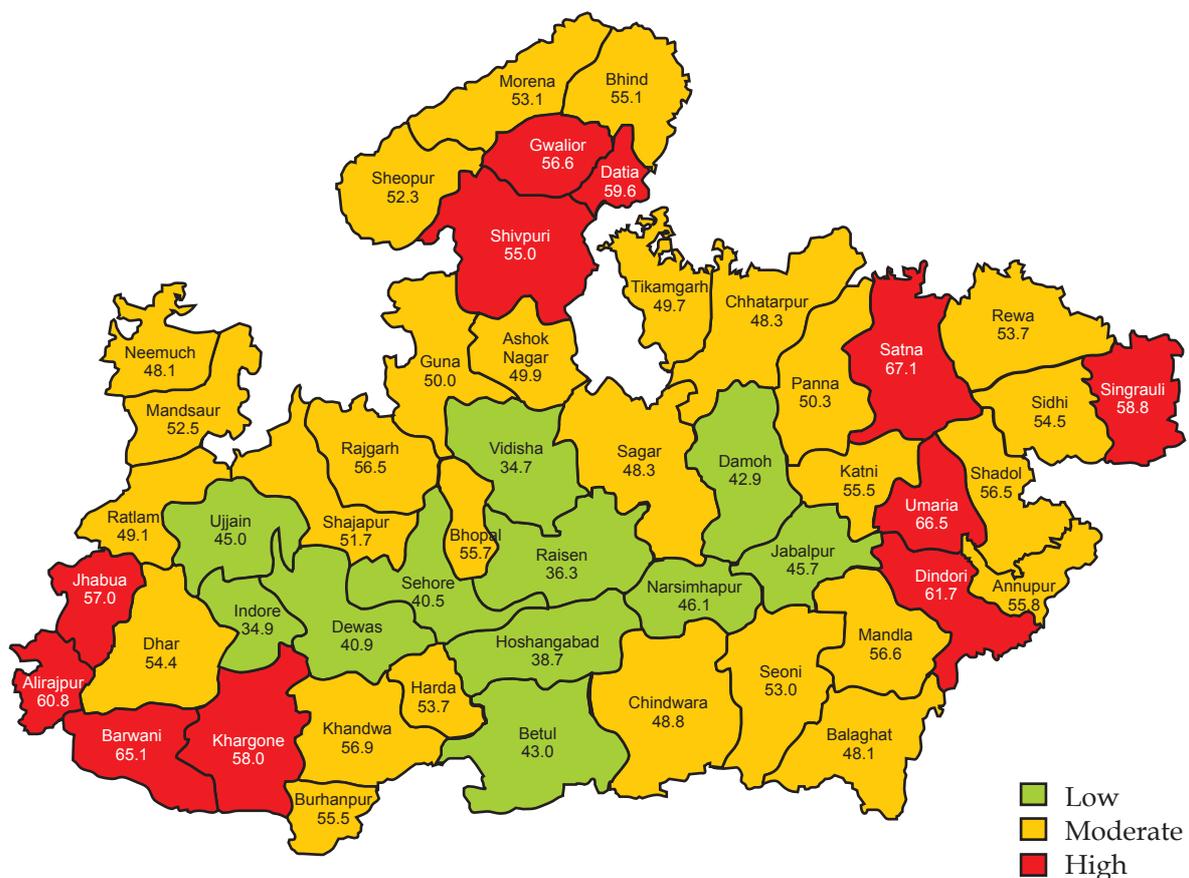
About one third of children (32.5%) were suffering from morbidities such as fever, ARI and diarrhoea during the preceding fortnight. The magnitude was relatively higher among 6-23 months children, which tended to decrease with increase in age.

## Nutritional status

The overall prevalence of undernutrition (<Median -2SD) i.e. underweight, stunting and wasting was 52%, 49% and 26% respectively. The prevalence of underweight and stunting tended to increase with age from 29% and 18% respectively at 5 months of age to 54% and 56% respectively between 36-47 months of age.

The study revealed that a significant association between nutritional status and different socioeconomic variables. The prevalence of underweight and stunting was significantly ( $p < 0.01$ ) associated with community, type of family, family size, literacy status and occupation of parents, extent of land holdings, per capita income, type of house, type of cooking fuel used, sanitary latrine, electricity and separate kitchen. The prevalence of wasting was significantly ( $p < 0.01$ ) associated with community, literacy status and occupation of parents, extent of land holdings, per capita income, type of house, source of drinking water, type of cooking fuel used, sanitary latrine, separate kitchen and presence of morbidity. Mapping of districts was done to know the district with high, medium and low prevalence of underweight as given in Fig 1. Red coloured districts indicate high prevalence, yellow indicates low and green normal.

**Fig.1 Madhya Pradesh map showing district-wise prevalence of underweight**



The study highlights the need to impart health and nutrition education to the pregnant and lactating mothers through effective IEC activities with emphasis on infant and young child feeding practices. The existing national nutrition intervention programmes such as supplementary feeding under ICDS and massive dose of Vitamin A supplementation have to be strengthened further. The poverty alleviation programme and other income generating activities may be strengthened to improve the household food security.

## 2 Nutritional and health status of street children in Hyderabad

Asia is known to have a large number of street children. At least 18 million children live or work on the streets of urban India. A majority of these children find themselves in the vicious cycle of crime, prostitution, gang related violence and drug trafficking. It is estimated that India has about two million street children mostly living in high-risk slum areas in the cities. Most of these children are sexually active from a young age. Many street children start their sexual careers before the age of 11 and soon start engaging in high-risk sexual behaviours with distressing frequencies. Estimates suggest that about 0.5% of this population becomes newly infected with HIV every year. Half of all children in India are malnourished, but in the case of street children, this number is much higher. According to the study by Rita Panicker, approximately 18 million children work or live on streets and a high percentage of them are sexually active in India.

Hyderabad is one of the fastest growing metropolitan cities in India. The proportion of street children is on the rise. No study has been carried out to assess the nutritional and health status of children so far in Hyderabad. Therefore, it has been proposed to study health and nutritional problems and prevalence of HIV/ AIDS among street children in Hyderabad.

### OBJECTIVES

**General objective:** To study nutritional and health status of street children in the twin cities of Hyderabad and Secunderabad.

**Primary objective:**

To assess Nutritional status by anthropometric indices

**Secondary objectives:**

1. To assess the prevalence of clinical signs of nutrition deficiency among street children in Hyderabad
2. To assess the prevalence of anaemia among these children
3. To assess knowledge and practices about HIV/ AIDS and STIs

### METHODOLOGY

**Study design:** A cross sectional study.

**Sample size:** Assuming 60% prevalence of underweight, 95% confidence interval and 6% absolute precision, sample required was 260 and approximately 300, would be covered.

**Sampling frame:** Sampling frame was developed by collecting details of all the NGOs and government institutions working for street children in Greater Hyderabad Municipal corporation (GHMC) areas and information was obtained from them about the site, address and number of street children at the particular site (min and max).

## **Inclusion criteria**

- ✍ Children between 8-18 years of age, living on the streets of GHMC areas
- ✍ Children who have minimal or no contacts with family

## **Exclusion criteria**

Children who are institutionalized and/or living in hostels.

**Data Analysis:** The data collected was scrutinized and entered into the computer, analysis was carried out by using SPSS Windows version 17.0. Descriptive analysis was done. Proportion test was also carried out to study association.

## **Anthropometry**

Mean heights and weights were presented according to age & sex. The nutritional status of children was assessed using BMI age and gender specific BMI centiles and height for age by using WHO standards (WHO).

## **Salient findings**

A total of 305 children were covered, age ranging from 8-18 years. Mean age of the children was 14.5 years (SD 2.4 yrs). A majority of them (75.7%) belonged to Hindu religion, followed by Muslim (18.0%). About 23% were illiterate, while 28-29% each were studied up to primary and upper primary. About 18% had SSC education. Majority of the children (63%) belonged to the state of Andhra Pradesh. About 12% children were orphans, 13% had only mothers, 7% had father only, while 69% had both the parents. About 39% of fathers and 51% mothers of street children were illiterate, while 18% children were not aware of literacy status of parents. A majority of children were living either at railway platform (28.9%) or on the street (28.9%).

The important reasons for being street children were either to earn money (57.4%) or to escape from family problems (19%) and 12% were orphaned. About 24% were working in hotels, 16% were working in road side stalls, 14% were rag pickers, 9% were engaged in collection of water bottles in the trains and 4% were beggars while 26% were not doing any work.

About 24% were smokers, 14% were consuming alcohol, 4% were smoking Ganja, while 5% were inhaling whitener and 35% were consuming other tobacco products such as *ghutkha*, tobacco etc. Poverty was an important reason for being on streets (86%), while 34% stated lack of accessible resources for being on the street. Only 14% children (>12 years) had sexual intercourse, mostly with street girls or peers.

About 39% were aware of HIV/AIDS, of which 28% were aware of at least one route of transmission of HIV. About 18% had dental fluorosis, 2% each had bitot spots and angular stomatitis, while 14% children had skin problems such as dermatitis, eczema etc. More than 50% had bad oral hygiene.

The overall prevalence of thinness (<Median-2SD) was about 26% and was higher among 8-9 yrs children (45.5%) as compared to 14-18 years children (28%). The overall prevalence of stunting (<Median-2SD) was about 48% and was higher among 14-18 years children (51%) and <10yrs (54.5%) as compared to 10-14 years children (37%).

Blood for hemoglobin estimation was collected from 250 children. The overall prevalence of anaemia was 54% and was higher among 8-12 years children (60.5%) as compared with 12-14 years children (47.0%).

Interestingly, the study revealed that the prevalence of undernutrition and anaemia was similar to rural and tribal children; even it was lower than those children.

Overweight, obesity and associated non-communicable diseases are posing a grave public health problem. They are concerns even among the adolescents. The most significant long-term consequence of childhood and adolescent obesity is the persistence of associated health risks even during adulthood. Therefore, healthy eating practices and regular physical activity from childhood is vital as it has got a potential impact on the physical, psychological and social well-being of the children in the later stages of life. Schools continue to be the best places for initiating healthy lifestyles and adopting dietary habits. Further, schools are the appropriate places for children to develop, test and adapt healthy eating and lifestyles through interaction with teachers, peers and friends. In addition, schools have the potential not only to reach the students, but also the school staff, teachers, parents and community member at large. Thus, school forms the entry point for initiation of appropriate interventions.

A study (Phase -I) was carried out in the year 2006-07 with the support from WHO to assess the 'prevalence of overweight and obesity and its determinants among the urban adolescent children in the State of Andhra Pradesh'. The findings of this study were utilized for designing and implementing intervention strategies to prevent and control overweight/obesity among the adolescents on a pilot study basis in Hyderabad in Phase- II during 2008-2009. After the Phase-II study, it was observed that the knowledge levels in health and nutrition among adolescents in the intervention group increased significantly as compared to those in the control group. The results also revealed that physical activities such as regular participation in outdoor games and sports, household activities etc, also improved significantly among the adolescents of the intervention group and duration of television viewing also declined. The prevalence of overweight and obesity also marginally declined among adolescents of the intervention group compared to the control group. In order to continue the intervention and assess its impact on the adolescents, the current project was proposed as Phase-III of the study, wherein the already developed and tested multi-component intervention strategies (developed during the phase-II study) were used for reinforcement along with certain measures to create enabling environment in schools.

The following were the objectives of the study:

### OBJECTIVES

To assess the effect of health and nutrition education on the physical activities and lifestyle practices and body mass index (BMI) among 10-15 year children studying in private schools in urban areas of Hyderabad.

#### *Specific objectives*

1. To re-orient science and physical education teachers periodically on healthy diet, lifestyles, overweight/obesity and its consequences,
2. To assess the effect of interventions on mean BMI and prevalence of overweight/obesity among adolescents, and
3. To orient the District Educational Officers (DEOs) in the State of Andhra Pradesh by conducting State level workshop on "Healthy diet and lifestyles".

### METHODOLOGY

For this purpose, the study used the same cluster randomized design and data collection procedures as used in Phase -II. Multi-component interventions were created and healthy diet and

# iCommunity studies

nutrition education was provided to the intervention group, while for the control group, only education material (leaflets) on health and nutrition was provided. Teachers and parents were sensitized with nutrition and health issues and sensitizing sessions were carried out once in a month in the selected schools. Workshops were conducted for teachers, district and mandal level education officers.

## *Salient Findings*

A total of 318 adolescents were recruited for intervention by cluster randomization method, on which baseline data was collected, while the endline data was collected from 283 adolescents. For control group, a total of 291 adolescents were recruited by from an earlier cohort (that was selected using cluster randomization method), of them, 237 adolescents were available for end line data collection. It is in fact, the continuation of earlier cohort (2008-09) with a gap of one year. Multiple interventions were provided to the adolescents of intervention schools for a period of 8 months and only health and nutrition education material was provided to the adolescents of control schools.

The baseline survey revealed that the demographic profile of the adolescents in both control and intervention schools was more or less similar. However, regarding socio-economic status, the intervention group consists of higher proportion of literate parents, who were in services or had their own business compared to the control group. Anthropometric data suggests only modest or no change in the weight of the adolescents in the intervention group. The data when compared with the mean BMI or sum of skin folds of children in intervention and control groups, showed only marginal difference. Nearly, 92% in the control and 89.4% in the intervention group maintained status quo with regards to their anthropometric measures. Merely 3.8% in the control group and 4.2% in the intervention group gained weight and 4.2% in the control group and 6.4% in the intervention group lost weight, which was insignificant and demonstrates no impact of sustained nutrition education on the BMI percentiles before and after intervention in control and intervention group, over and above what was achieved during phase-II.

The results of the study also showed a significant improvement in knowledge on basic nutrition and health issues in the intervention group compared to control group. The impact was observed in knowledge associated with basic nutrition such as major energy sources from: carbohydrate (control: 34.6% vs. intervention group: 55.1 %,  $p<0.001$ ), nutrient that helps in body building: proteins (control: 33.8% vs. intervention group: 75.3%,  $p<0.001$ ), foods that give proteins (control: 46% vs. intervention group: 69.3%  $p<0.001$ ), foods that provide protective nutrients (control: 82.7% vs. intervention group: 88.7%,  $p<0.05$ ), foods that are rich in vitamin A (control: 53.2% vs. intervention group: 73.9%  $p<0.001$ ). Further, following intervention for 8 months, a significant improvement was also observed in knowledge on the subject of foods that are healthy and unhealthy such as consumption of energy dense foods leading to obesity ( $p<0.01$ ), variety of foods for a balanced diet ( $p<0.001$ ) and deep fried foods are not healthy ( $P<0.05$ ) in the intervention group compared to control group.

Subsequent to the intervention, a significant improvement in knowledge regarding reasons for overweight and obesity such as not consuming balanced diet ( $p<0.001$ ), watching TV ( $p<0.001$ ), hereditary ( $p<0.001$ ), mental stress ( $p<0.001$ ), frequent snacking ( $p<0.001$ ) leads to overweight was too observed in the intervention group compared to the control group. On the contrary, no significant change was observed in the practices of healthy lifestyle and physical activities, except significant improvement was observed in performing some physical activities such as walking ( $p<0.01$ ), cycling ( $p<0.01$ ) in the intervention group compared to control.

The present study demonstrates that there was significant improvement in the short-term outcomes such as knowledge and perceptions about healthy diet and lifestyles, but did not show

improvement in the long-term indicators such as behavior change such as practice of healthy lifestyle, physical activity and anthropometric and adiposity indices.

## **Re-orientation of educational officers**

In addition to the above, re-orientation of authorities of the educational institutions and sensitization of District Education Officers (DEOs) in the State of Andhra Pradesh were also carried through a workshop on nutrition and health education. The main objective of this approach was to bring awareness among the secondary audiences such as decision makers on the importance of practicing healthy dietary choices and lifestyles to prevent childhood obesity. Continuous educational sessions and collaborations are important to convince them to promote enabling school environment. Further, the information obtained through workshops, training programs encourage decision makers to restructure the school curriculum by increasing the number of physical education sessions and by including topics related to health and nutrition.

Given this background, a State-level Workshop for DEOs and MEOs of Andhra Pradesh was conducted at NIN, Hyderabad on 29-30<sup>th</sup> August 2011 with the following objectives:

1. To impart nutrition and health education and bring awareness on healthy diets and life styles to DEO's, DyEO's and MEO's attending the workshop.
2. To enhance knowledge on promotion of enabling school environment

Multiple health and nutrition related topics were presented by NIN scientists and group activities were also conducted in the workshop. The feedback was positive and participants felt that workshop was very informative. Some of them recommended presenting material in regional language is mandatory for future workshops, since most of the schools in rural areas teach in regional language. Some suggested conducting workshops for teachers was very informative as they would be equipped with nutrition knowledge and skills which in turn could be passed on to their students and staff as well. On the whole participants perceived that their knowledge on the subject of childhood obesity, its consequences and importance of healthy eating among children had improved and felt that workshop aided them with information to develop strategies to educate and train their staff to promote an enabling school environment.

On the other hand, the duration of workshop was only one and half day which was not enough to give comprehensive nutrition and health education. This requires considerable time, substantial funds and effective resource personnel to constantly educate and work with various decision makers from different sectors. This indicates the need for conducting cost effective workshop seriatim at the regional and mandal levels through utilizing locally available resources.

Based on the results of the intervention study, it is recommended that the duration of school-based nutrition education intervention should be continued for more than two years to observe significant changes in behavioral indicators such as anthropometric and adiposity indices. Further, it reiterates the need for nation-wide multi-component and multi-channel intervention programmes to be initiated with schools as entry points to prevent and control overweight and obesity among adolescents. In order to create enabling environments at schools to promote healthy eating and physical activities, teachers, school managements, district level education officers and policy makers should be re-oriented from time to time.

## II. BIostatistics and Bioinformatics

### 1 Factors influencing infant growth in vulnerable rural South Indian infants

More than 50% of the world's undernourished population live in India, yet evidence also suggests an emerging nutrition transition, resulting in a growing amount of the population being susceptible to overweight and associated chronic diseases. The Barker hypothesis suggests that children born with low birth weight undergo physical adaptations (endocrine, metabolic, & structural) that prioritize survival when the growing foetus is placed under pressure because of inadequate nutrition. Infants exposed to environments during pregnancy that put them at risk of malnutrition through decreased protein or energy intakes, but who later become exposed to more developed (transitioned) environments appear to be in the most danger of developing risk factors for chronic diseases (e.g. metabolic syndrome). This happens through insults that occur during critical windows for growth to alter body tissue structure and function. Such associations have been clearly demonstrated in Indian children born with low birth weight. Yajnik (2000) has identified a need for a better understanding of the factors that promote rapid growth in infants born with low birth weight to enable evidence based recommendations for appropriate growth trajectories for such infants. Such understanding is more likely to be developed from studies of low birth weight infants in developing countries where more infants are born low birth weight. However, the findings of such studies also have global relevance to the understanding of early infant growth and its association with disease risk.

#### OBJECTIVES

To assess whether there are differences in the factors (child, maternal, household and community) predicting growth patterns (slow, normal or rapid) between rural Indian infants born with normal birth weight and to well nourished mothers compared to those born with low birth weight and/or to underweight mothers.

#### Data used

1. Indo-US Collaborative study 'Efficacy of an integrated feeding and care intervention among 3 to 15 months old rural children in Andhra Pradesh, India', which is a longitudinal study designed to investigate the role of infant feeding and care practices on infant development from birth to 15 months of age (n=600). The study recruited all infants born across three ICDS Project Areas covering 60 villages in the rural Nalgonda district of Andhra Pradesh in India, between Sep 2005-April 2007.
2. A double-blind randomized, controlled clinical trial of Zinc supplementation to full-term infants in Hyderabad urban slums. Cohort followed from 4 to 24 months (n=427).

#### Data Analysis

The longitudinal study design and the data on all elements of the UNICEF conceptual framework for care for nutrition offer an opportunity to construct a multi-level analysis of the role of maternal, household and community level factors and their contribution to infant growth in infancy. This sample provides a unique opportunity to study these associations because over 40% were born vulnerable i.e., low birth weight (<2.5kg) and/or to an underweight mother (BMI <18.5). It was therefore able to assess potential differences in the role of our key variables of interest on children's growth for these two susceptible groups.

Length and weight trajectories are modelled using multi level models (MLM) with SPSS 15.0 and MLWin software. MLM is appropriate for data with a nested structure, such as repeated-measures in which several individual measurements (follow-ups) are nested within individuals (children). MLM allows the separation of the within-child (Level 1) and between-child variance (Level 2) in the outcome under study.

## RESULTS

The prevalence of children in each of the four distinct groups relating to the four BMI/ birth weight groups has been estimated (low birth weight (< 2.5Kg) and low BMI mother (<18.5), low birth weight and normal BMI mother, normal birth weight and low BMI mother, and normal birth weight and normal BMI mother). Table 1 shows that for both the INDO-US and the Zinc studies over 40% of infants were either born low birth weight or to a mother with a low BMI.

As well as establishing the appropriate shape of curve to fit to the Zinc / INDO-US studies' data, it has been possible to model the association of the key maternal, infant and socio-demographic predictors of infant growth with the Zinc study as well as Infant feeding data (INDO\_US). Intervention groups, maternal age, education and occupation of both parents and gestational age were not associated with growth. Variables associated with growth were age, age squared, gender, and heights and weights of the mother, morbidity score (Indo-US study only for weight gain), and possession of assets (Indo-US study only for height) in addition to the grouped birth weight and BMI variable. For the main variable of interest the reference group were the normal birth weight (> 2.5kg) and normal BMI group (>18.5). Compared to this group those who were born low birth weight and low BMI maintain a significantly shorter height (2.94cms on average in the Zinc study and 1.81 in the Indo-US study).

However, those with a low birth weight and normal BMI were also significantly shorter although less so (2.01 and 1.21 cms on average in the Zinc and INDO-US studies respectively), as were those who were normal birth weight and low BMI compared to the normal birth weight (0.51 cms on average in zinc study for height only) and normal BMI group (Fig 2 and 3 & Tables 2a/2b). The pattern of findings for weight were similar except where previously indicated. Thus we find that infants born either of low birth weight or with a low BMI have a significant disadvantage in growth in height and weight, although the largest disadvantage is for those born low birth weight.

The use of MLM allowed us to ascertain that the between individual variation is significant for both studies and for both weight and height outcomes. For instance, in the case of height for the zinc study, it was estimated as 3.5 (se = 0.35) and the between measurement occasions within individuals was significant and estimated as 2.96 (se = 0.09). The significance of the between individual variation reveals the importance of taking account of the clustering of individual measures of growth within individuals. A single level model would be inappropriate in this case and would likely result in biased estimates of the standard errors of the parameter estimates and potentially incorrect conclusions regarding statistical significance. The negative sign of the covariance term in some of our models also reveals that infants with increased intercepts (starting values for height or weight), have less steep slopes on their growth curves.

This was only statistically significant in the case of height for the Indo-US study. For these infants this was positive as it shows that infants in this sample are showing a tendency towards catch-up growth when they start out life at a low height value. However, the only other significant covariance parameter was for weight for the zinc study and this value was positive indicating that the infants who start at higher weights also have the tendency to gain more weight. However, among infants from the zinc study rapid growth, i.e., weight and height, was not observed in the more vulnerable infants who start with the lower weight and height values. The models also show

significant variation in the intercept and the slopes between individuals, which was not surprising given the variation in human growth starting points and trajectories that are known between individuals.

**Table 1. Distribution (%) of Infants by birthweight and Maternal BMI**

Birthweight & Maternal BMI	INDO-US Study	Zinc Study
Low birth weight (<2.5kg) and low BMI mother (<18.5)	5.5	11.8
Low birth weight (<2.5kg) and normal BMI mother (≥18.5)	9.8	8.3
Normal birth weight (≥2.5kg) and low BMI mother (<18.5)	28.7	29.6
Normal birth weight (2.5kg) and normal BMI mother (≥18.5)	56.1	50.3

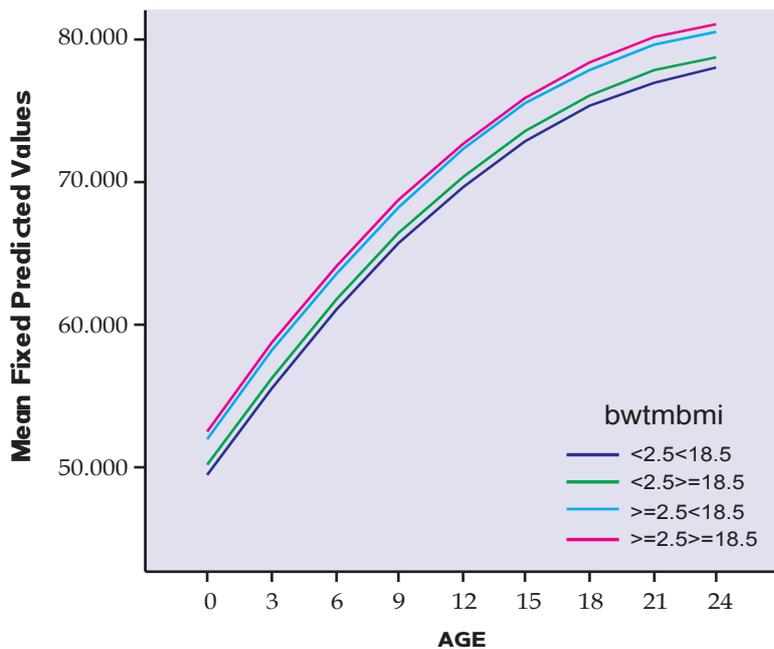
**Table 2a. Results for Level 1 (within-child) and Level 2 (between-child) regressions including time-varying variables and fixed child and maternal variables**

Fixed Effects	Linear growth		Weight gain	
	β	SE	β	SE
Intercept	50.781*	0.697	3.356*	0.138
Age	2.172*	0.016	0.545*	0.005
Age <sup>2</sup>	-0.041*	0.0006	-0.013*	0.0002
Sex(Ref : Female)	-0.993*	0.211	-0.291*	0.053
Maternal Ht/Wt(Ref:≥145cm/≥45Kg)	1.479*	0.346	0.207*	0.069
Low BWt,Low Maternal BMI (Ref: Both Normal)	-2.943*	0.343	-0.715*	0.098
Low BWt,Normal Maternal BMI (Ref: Both Normal)	-2.004*	0.404	-0.606*	0.099
Normal BWt,Low Maternal BMI (Ref: Both Normal)	-0.512*	0.245	0.032	0.077
Supplement days 7m (Ref:<6m)	-0.680	0.352	-0.047	0.087
Supplement days 8m(Ref:<6m)	-0.201	0.308	0.035	0.077
Supplement days ≥8m(Ref:<6m)	-1.454*	0.252	-0.190*	0.063
<b>Random Effects</b>				
<b>Between individuals</b>				
Initial status, $\sigma^2$	3.474*	0.345	0.130*	0.020
Age $\sigma^2$	0.012*	0.001	0.001*	0.0002
Covariance <sup>2</sup> ( $\sigma_{01}$ )	-0.006	0.016	0.012*	0.001
<b>Within individuals</b>				
Residual, $\sigma^2$	2.968*	0.085	0.371*	0.010

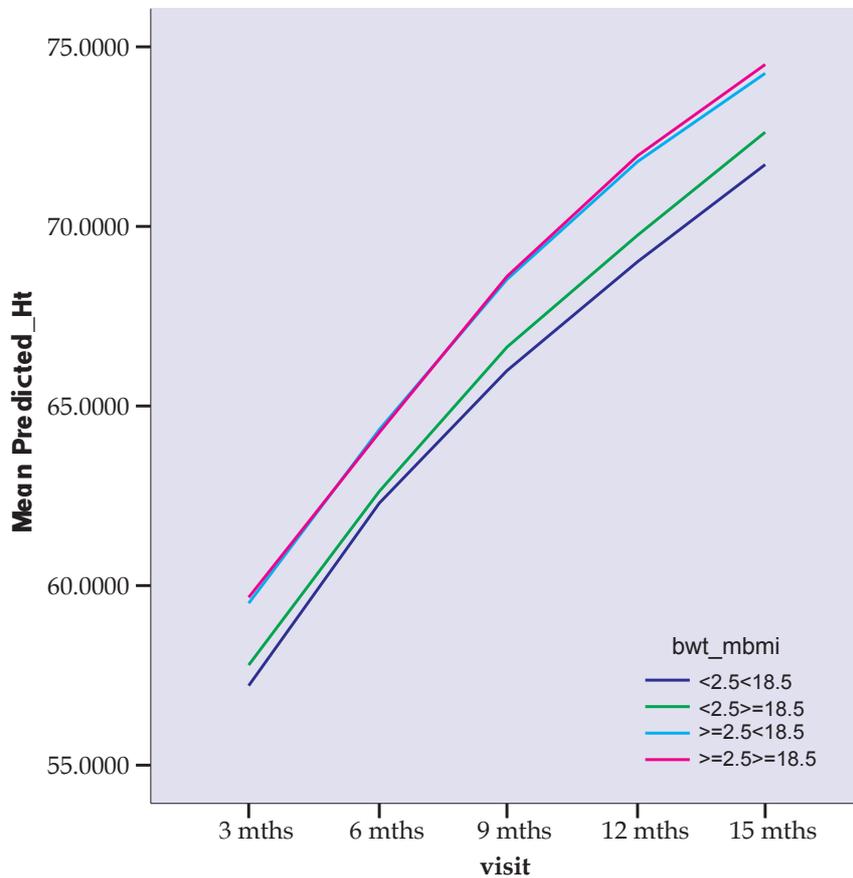
**Table 2b. Results for Level 1 (within-child) and Level 2 (between-child) regressions including time-varying variables and fixed child and maternal variables (INDO-US Study)**

Fixed Effects	Linear growth		Weight gain	
	$\beta$	SE	$\beta$	SE
Intercept	51.627*	0.714	4.696*	0.213
Age	2.040*	0.039	0.478*	0.014
Age <sup>2</sup>	-0.045*	0.002	-0.013*	0.0007
Sex(Ref:Female)	-1.329*	0.189	-0.435*	0.074
Maternal Ht/Wt(Ref:>=145cm;>=45Kg)	1.904*	0.306	0.292*	0.091
Low BWt,Low Maternal BMI (Ref: Both Normal)	-1.814*	0.420	-0.719*	0.172
Low BWt,Normal Maternal BMI (Ref: Both Normal)	-1.213*	0.349	-0.464*	0.141
Normal BWt,Low Maternal BMI (Ref: Both Normal)	-0.115	0.217	-0.112	0.101
Morbidity Score	-0.030	0.035	-0.065*	0.012
Assets	0.020*	0.006	0.003	0.002
<b>Random Effects</b>				
<b>Between individuals</b>				
Initial status, $\sigma^2$	3.510*	0.404	0.476*	0.055
Age $\sigma^2$	0.015*	0.003	0.002*	0.0003
Covariance <sup>2</sup> ( $\sigma_{01}$ )	-0.063*	0.027	-0.003	0.003
<b>Within individuals</b>				
Residual, $\sigma^2$	0.954*	0.063	0.115*	0.008

**Fig 2. Growth trajectories of infants in the Hyderabad Zinc study born into four distinct groups (low birth weight (< 2.5Kg) and low BMI mother (<18.5), low birth weight and normal BMI mother, normal birth weight and low BMI mother, and normal birth weight and normal BMI mother)**



**Fig 3. Growth trajectories of infants in the Indo-US Infant feeding study born into four distinct groups (low birth weight (< 2.5Kg) and low BMI mother (<18.5), low birth weight and normal BMI mother, normal birth weight and low BMI mother, and normal birth weight and normal BMI mother)**



## SUMMARY

Findings were revealed that the combined effects of maternal BMI and low birth weight had the most negative effects on the growth trajectories of infants in early life, suggesting that, these two measures could be used in combination to target the most at risk infants for growth monitoring and intervention.

### III. CLINICAL STUDIES

#### 1 Nutritional challenges, abdominal adiposity and type 2 diabetes in Indians

The thrifty phenotype, adapted to nutritional shortages *in utero* and early life is predisposed to store fat whenever available leading to obesity. Rapid urbanization and migration with consequent changes in diet, exercise and increasing obesity making them vulnerable to developing insulin resistance, diabetes and CHD as a likely consequence of greater abdominal adiposity.

This study was done in collaboration with London School of Hygiene and Tropical Medicine (LSHTM) UK, funded by Wellcome Trust. The aim of the study 'Hyderabad Nutrition Trial (HNT)' was to examine the effect of nutritional shortage/supplementation in early life and adulthood on the amount and distribution of body fat and the development of type 2 diabetes and coronary disease, in two cohorts which were followed earlier. Association between total body fat and abdominal adiposity with type 2 diabetes, insulin resistance, impaired fasting glucose (IFG) and coronary disease were also studied.

#### HYPOTHESES

Nutritional challenges during intra-uterine life and early infancy predisposes to abdominal obesity, diabetes and coronary heart disease in later life.

1. Children who received nutritional supplementation in early life have less abdominal adiposity, insulin resistance and presymptomatic vascular disease than children who were not supplemented.
2. Urban migrants have more abdominal adiposity, diabetes and CHD than their rural siblings.
3. Abdominal adiposity is more strongly associated with markers of diabetes and CHD than total body fat.

#### OBJECTIVES

1. To identify predictors of total body fat and abdominal adiposity, in populations who received nutritional supplementation in early life and rural-urban migrants.
2. To assess the association between nutritional supplementation in early life with the development of diabetes and coronary disease.
3. To assess the association between rural-urban migration with the development of diabetes and coronary disease.
4. To assess the association between total body fat and abdominal adiposity with type 2 diabetes, insulin resistance, impaired fasting glucose (IFG) and coronary disease.

#### Sample size :

About 525 per group was arrived, with a standard deviation (SD) of 2 in BMI and expecting a difference of 0.2 in SD in the between groups with 90% power at 5% level of significance.

## iii Clinical studies

### Study participants

The HNT started in 1987-90 as a trial of supplementation of pregnant mothers' and their infants (n=2601) in rural areas, comparing 15 villages receiving nutritional supplementation and 14 villages as controls.

The Indian Migrant Study in Hyderabad was nested within a Cardiovascular Disease Risk Factor Study in four Indian cities (Bangalore, Lucknow, Nagpur and Hyderabad). Each participant was asked to identify one non-migrant sibling of the same sex and closest to them in age. Detailed information was collected on diet, activity, anthropometric variables, diabetes and IFG. The cohort study in Hyderabad includes 818 urban dwellers, working in one factory, with 818 rural siblings. 56% of the cohort is male, and the majority are mostly aged 35-55 years (mean age 44).

**Exclusion Criteria:** All pregnant women were excluded from the study.

### METHODOLOGY

1. Subjects from each group of supplemented and un-supplemented villages of an earlier trial were recruited for the study as well as from the Hyderabad arm of the Indian Migration Study.
2. DXA scanning was done to measure bone parameters, total body fat as well as regional adiposity. Anthropometric measures were also used for measuring body composition.
3. Systolic and diastolic blood pressure were measured for all subjects. The augmentation index (AIx) and pulse wave velocity were measured for arterial stiffness and carotid intima media thickness (IMT) was assessed using an ultrasonograph equipped with a 7.5-MHZ linear type B-mode probe as a subclinical measure of atherosclerosis.
4. Assessment of dietary intake, physical activity was done by a questionnaire to all participants. Dietary intakes were assessed through a semi-quantitative food frequency questionnaire. Information on standard of living index (asset ownership, education and employment).
5. Venous blood samples were collected after a 12 hour fast, separated and stored at -20°C locally for assays at NIN. Oral glucose tolerance test was done in all subjects and insulin was estimated by radio-immunoassay. HDL and total cholesterol, triglycerides, plasma glucose, vitamin D and micro-albuminuria were being estimated with standard methods.

### RESULTS

Analysis for validation of Dual-energy X-ray absorptiometry (DXA) with Magnetic resonance imaging (MRI) has shown that DXA measures of abdominal fat are suitable for use in an Indian population. Intra-observer reliability was high (ICCs all >0.9) (Table 3).

Table 3

Parameters	Intervention n=736	Control N=709	Mean difference (95% CI)	P value
Height (cm)	161.9	162.1	0.2 (-1.4 to 1.8)	0.825
Weight (kg)	51.2	51.5	0.4 (-1.3 to 2.0)	0.659
BMI	19.4	19.5	0.1 (-0.4 to 0.6)	0.451
Total lean mass (kg)	38.85	39.71	0.85 (-0.66 to 2.35)	0.259
Appendicular skeletal mass	17.54	18.03	0.50 (-0.3 to 1.3)	0.228
Fat free mass index	14.62	14.90	0.28 (-.07 to 0.64)	0.112
Grip strength (kg)	28.9	29.6	0.6 (-1.1 to 2.4)	0.468
Total body fat (kg) #	9.1	8.7	1.04 (0.97 to 1.11)	0.328
Body fat % #	18.0	17.1	1.04 (0.99 to 1.09)	0.124

### iii Clinical studies

The difference between DXA and MRI measures of abdominal fat was not significant with mean difference L1-L4 region being 12g, ( $p=0.62$ ) and mean difference L2-L4 region being 21g, ( $p=0.32$ ). However, on log transformation there was evidence that DXA overestimated abdominal fat compared to MRI in leaner participants with the least abdominal fat and similarly underestimated abdominal fat compared to MRI in participants with the most abdominal fat.

Preliminary analysis of the data examining the influence of early life nutrition intervention on LBM development of young adults had shown that modest protein-calorie supplementation in early life was not associated with higher LBM in this sample of rural young adults. Higher percentage of subjects in the control area were illiterate which was significant. (25 vs 16,  $p<0.001$ ). 27.5% of all subjects were vitamin D deficient. ( $<20\text{ng/ml}$ ) and they were not significantly different. Vitamin D levels, manual occupation and dietary protein intake were significant determinants of lean mass in these individuals. ( $p$  value 0.024, 0.002 and  $<0.001$  respectively).

## IV. MICROBIOLOGY AND IMMUNOLOGY

1

### Molecular characterization of reshuffled bile salt hydrolase (Bsh) and effect of dietary inclusion of Bsh<sup>+</sup> and Bsh<sup>-</sup> indigenous probiotic *Lactobacillus plantarum* strains of human origin on cholesterol metabolism of rats

The World Health Organization (WHO) predicts that by the year 2020 up to 40% of all deaths will be related to cardiovascular diseases or disease of the heart. Although pharmacologic agents (3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors or drugs) are available to treat this condition, they are often suboptimal and expensive and may have unwanted side effects. Some studies have shown cholesterol lowering ability of bile salt hydrolase active probiotic bacterium or products containing them through host bile salt metabolism. The deconjugation of bile acids is the primary mode of hypocholesterolemic action. An alternative mechanism is the direct inhibition of HMG-CoA reductase; possible inhibitors include hydroxymethylglutaric acid, orotic acid, and uric acid from lactic acid fermentation. With the completion of some probiotic genome projects, analysis of sequenced probiotic strains reveal that many bacteria possess more than one Bsh homolog, but all are not effective in reducing cholesterol. Therefore, it is proposed to characterize the bile salt hydrolases from two *Lactobacillus plantarum* strains (Bsh gene sequences of these two strains were reshuffled by the insertion of transposon DNA, as confirmed by previous studies) and to elucidate the exact role of Bsh in controlling hypercholesterolemia.

#### OBJECTIVES

1. To express and characterize Bsh gene from indigenous *Lactobacillus plantarum* strains of Indian origin.
2. To examine the effect of Bsh active *L. plantarum* strains on serum/plasma cholesterol levels and gastrointestinal colonization in rat model.

#### METHODOLOGY

*L. plantarum* strains RK21, RK37 and RK83 were isolated from human faeces and identified at genus, species & strain levels by specific primed PCR assays developed in the laboratory and were employed as the alternatives of Lp91, Lp20 and Lp77.

#### Genetic Engineering of Reshuffled *bsh* gene

- a) PCR amplification of full length *bsh1* and reshuffled *bsh1* gene using degenerate primers based on the conserved *bsh* gene sequences obtained from previous studies and from the database.
- b) Site specific engineering of reshuffled *bsh* gene using primers based on their nucleotide sequences.
- c) Cloning of the purified PCR amplified product in *E. coli* DH5 $\alpha$  using pJET<sup>TM</sup> (cloning vector, Fermentas) and confirmation of inframe orientation of insert in the recombinant clones by PCR, restriction digestion.
- d) Characterization and analysis of the nucleotides and deduced amino acid sequences and alignment with the sequences available in database for homology and relatedness.

#### Expression, purification and characterization of recombinant Bsh

- a) Screening of recombinant clones with antibiotic markers, PCR and restriction digestion for correct frame of orientation of *bsh1* insert.

- b) *Bsh1* active inserts from reshuffled *bsh* of *L. plantarum* RK83 and *L. plantarum* RK37 were cloned into pET28b (+) vector and introduction of the recombinant construct and its expression in *E. coli*, BL-21 (DE3). Recombinant clones were screened with antibiotic markers, PCR, restriction digestion and nucleotide sequencing for correct frame of orientation of genetically engineered *bsh* insert along with *bsh1*.

Cholesterol lowering ability and gastrointestinal colonization potential of probiotic *Lactobacillus plantarum* strains (selected Bsh<sup>+</sup> and Bsh<sup>-</sup> *Lactobacillus plantarum* strains) were tested in rat model.

**In vivo studies:** Hypocholesterolemic effect of Bsh<sup>+</sup> and Bsh<sup>-</sup> *Lactobacillus plantarum* strains was evaluated in rat animal model. Assessment of *in vitro* probiotic attributes of selected *Lactobacillus* strains were done.

- Blood samples of each group of animals were analyzed for serum total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. Serum total-cholesterol values were compared with control groups to determine the hypocholesterolemic effect of probiotic strains.
- Animals were euthanized for liver and adipose RNA isolation. The RNA samples were subjected to cDNA synthesis for RT-PCR analysis. Specific primers were used to determine the expression of LDL-receptor, HMG-CoA reductase, 7 $\alpha$  dehydroxylase in respect to GAPDH.
- Caecal contents were analyzed to determine microbiological counts like total plate counts, *E. coli*, Coliform, *Bifidobacterium* and *Lactobacillus* counts. DNA fingerprinting of Caecal lactobacilli of each groups using RAPD-PCR technique were carried out.
- DNA fingerprinting of faecal lactobacilli of each groups was carried out using RAPD-PCR technique.

## RESULTS

Cloning of *bsh* genes to identify the functional gene.

### 1. Bsh-PCR assay for *L. plantarum* strains identification

All the three Bsh<sup>+</sup> *L. plantarum* strains produced an amplicon ~1.0 kb size which represented the full length *bsh* gene. On the other hand, strains RK83 and RK37 with diminished Bsh activity which was initially thought to be devoid of *bsh* locus unexpectedly produced a distinct ~2.0 kb amplicon instead of the expected 1.0 kb product.

### 2. Sequence analysis and characterization of bile salt hydrolases

Nucleotide sequence of 876 bp long GE-*bsh* gene exhibited high level of sequence similarity to previously published *bsh* gene of reference *L. plantarum* strains, while *bsh* gene sequence of *L. rhamnosus* GG and *B. breve* ATCC15700 exhibited 24.6 and 36.2 percent of sequence similarity with *L. plantarum* Bsh1 at amino acid level.

### 3. PCR amplification of active *bsh* gene fractions from reshuffled Bsh

PCR amplicons of ~ 260 bp and 620 bp were amplified from genomic DNA of RK83 and RK37 by using the primers LpBsh1F/Lp20Bsh1R and Lp77Bsh2F/LpBsh1R respectively. Normal Bsh amplicon of ~ 1 Kb from RK11 and Bsh active amplicons of ~ 260 bp and ~620 bp from RK83 and RK37 were electrophoresed and extracted from the agarose gel by using Gene Elute™ gel extraction kit (Sigma).

### 4. Cloning of purified *bsh* amplicons into pJET™ cloning vector

Purified *bsh* amplicons of *L. plantarum* RK83 and RK37 (~260 bp and ~620 bp) and RK11 (~1.0 kb) were ligated into pJET™ cloning vector (Fermentas) and transformed into *E. coli* DH5 $\alpha$ . In

addition, *bsh* genes from *L. rhamnosus* GG, *L. fermentum* NCDC141 and *B. breve* ATCC15700 were also cloned successfully in this study. Nucleotide sequences of *bsh* inserts cloned into pJET vector were determined by custom nucleotide services (Chromous Biotech Pvt Ltd.).

#### 5. Sub-cloning of *bsh1* into pET28b (+) expression vector

Recombinant plasmids carrying *bsh* active fractions of RK83 and RK37 and *bsh1* insert of RK11 were digested by NdeI+HindIII, HindIII+XhoI and NdeI+XhoI restriction enzymes and separated on agarose gel by electrophoresis. Moreover, *bsh* genes of *L. rhamnosus* GG and *B. breve* ATCC15700 were also cloned into PET28b(+) expression vector successfully. Further, nucleotide sequences of GE-Bsh were determined using the custom services (Chromous Biotech Pvt. Ltd.) and submitted to the NCBI as a synthetic construct.

#### 6. Expression and purification of GE-Bsh and recombinant *bsh1*

Expression of recombinant Bsh proteins (rBsh and Nus-Bsh) was induced with 0.1 to 1.0 mM IPTG at 25°C - 37°C for 2-6 h and SDS-PAGE analysis revealed the appearance of ~37 kDa (GE-Bsh) and ~40 kDa (rBsh1) protein bands just after 2h of IPTG (0.1mM) induction at 22°C of recombinant *E. coli* clones. However, maximum level of recombinant protein expression was achieved after inducing the recombinant *E. coli* lag phase grown cells with 0.1mM IPTG for 4 h at 22°C. Enzymatic activity of GE-Bsh can be recovered by further optimization of GE-Bsh heterologous expression to preserve its cytoplasmic solubility. Predicted pI of GE-Bsh was 5.9 while rBsh1 had theoretical pI of 4.92.

#### 7. Assessment of *in vitro* probiotic attributes of selected *Lactobacillus* strains

Candidate *L. plantarum* strains RK21, RK83 and RK37 were tested for their ability to hydrolyze conjugated bile, to remove cholesterol *in vitro*, to survive under simulated gut physiological solutions. Obtained results clearly indicated that RK83 and RK37 exhibited negligible Bsh activity compared to RK21 while no significant difference was observed among their acid and bile tolerance abilities ( $P > 0.05$ ).

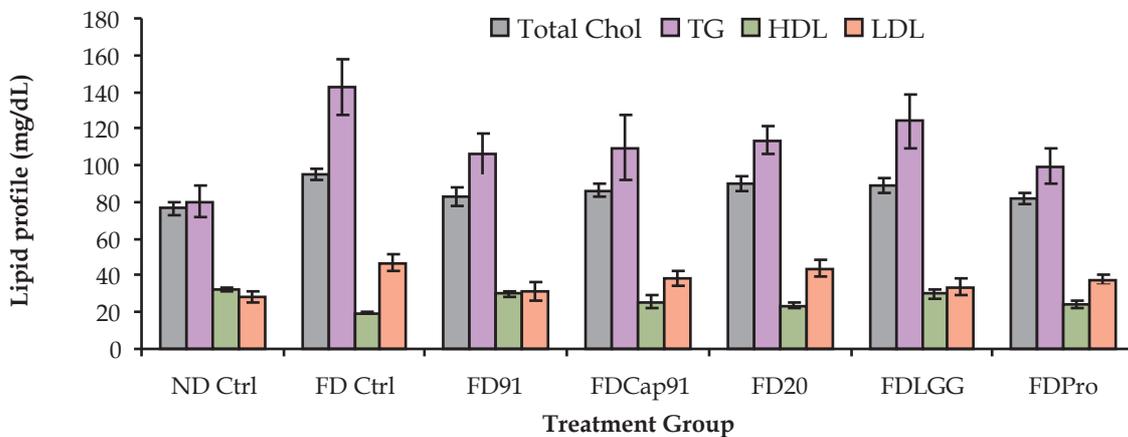
#### 8. *In vivo* cholesterol lowering ability of Bsh active probiotic strain

- a. Compared with HD control group, serum total cholesterol concentrations were reduced by 13-30% among all probiotic treatment groups. Minimum serum cholesterol decrease of 13% was recorded in HD20 group animals while maximum serum cholesterol reduction was noticed in HD91 group. In a similar way, LDL-cholesterol levels were also reduced significantly ( $P < 0.05$ ) in all probiotic treatment groups compared to HD control group after 28 days of experimental period while non-significant ( $P > 0.05$ ) decrease in triglyceride levels was also recorded after probiotic dietary treatment. Atherogenic index for probiotic treatment groups decreased significantly ( $P < 0.05$ ) after 28 days of treatment as compared to HD control groups (Fig 4).
- b. Serum total-, HDL-, LDL-, VLDL-cholesterol and triglycerides levels were significantly ( $P < 0.05$ ) affected by probiotic supplementation among animals receiving fat-enriched diet. Supplementation of Bsh active strain RK21 resulted in 13.68% decrease of serum total-cholesterol compared to 5.26% reduction by Bsh inactive strain RK37. However, maximum reduction of 30% in triglycerides levels was recorded in FD Pro group animals following with 25% triglyceride reduction among FD91 animals. HDL-cholesterol levels were significantly ( $P < 0.05$ ) enhanced in all probiotic groups while maximum increase was observed in FD91 and FDLGG groups. However, LDL-cholesterol levels were maintained significantly among all probiotic treatment groups with exception of HD20 group (Fig 5).
- c. Expression levels of cholesterol metabolism related genes i.e. *hmgr* (HMG-CoA reductase), *ldlr* (LDL receptor) and 7 $\alpha$ -hydroxylase, were affected significantly by probiotic supplementation.

**Fig. 4. Multiple sequence alignment of GE-Bsh with other Bsh proteins and their predicted structures.** The introduction of gaps is indicated by dashes. Black boxes indicate conserved amino acids residues for all sequences. Grey boxes represent identical amino acids residues. Lp, *L. plantarum*; Lrham, *L. rhamnosus*; Ljon, *L. johnsonii* and Bbrev, *Bifidobacterium breve*; PVA, *Pencillin V acylase* from *Bacillus sphaericus*. (Consensus, consensus sequences present in alignment; identity, sequence identity graph of alignment).



**Fig. 5. Changes in mean ( $\pm$ SEM) serum lipid profiles ( $P \leq 0.05$ )**



### 9. Role of Bsh in gastrointestinal persistence and colonization

- a. Caecal and faecal bacterial quantification revealed a significant increase in *Lactobacillus* count in all probiotic treatment groups compared to control groups during the experimental period.
- b. RAPD analysis of caecal and faecal *Lactobacillus* isolates recovered from Bsh active (RK21) group animals, using primers OPAA-02, OPAP-01 and OPBB-01 reflected significant similarity of 90-100% with their parent strain. However, Bsh inactive RK37 strain could not be traced in caecal and faecal samples of RK37 group of animals.

### CONCLUSIONS

1. GE-*bsh* gene constructed from the disrupted *bsh1* gene sequence of *L. plantarum* strains RK37 and RK83 exhibited high level of sequence similarity to previously published reference *bsh* gene sequences. Nucleotide and amino acid sequence of the GE-Bsh has been submitted to the NCBI as a synthetic construct. PCR analysis confirmed the presence of intact *bsh2*, *bsh3* and *bsh4* gene in RK37 and RK83 genome. Hence, it can be concluded that Bsh1 present in *L. plantarum* genome is mainly responsible for its bile salt hydrolyzing ability while ecological significance of the presence of *bsh2*, *bsh3* and *bsh4* is still not very clear.
2. Bile salt hydrolase active (Bsh<sup>+</sup>) *L. plantarum* strain RK21 significantly reduced serum cholesterol (Total-, LDL-, VLDL-) and triglyceride levels in comparison to Bsh inactive *L. plantarum* strain Rk37.
3. Bsh active strain RK21 colonized successfully into the cecum and large intestine of the respective animal groups.
4. Bile salt hydrolase activity helps the lactobacilli to colonize in rat gut and hence can be considered as the probiotic marker.

Strain RK37 and RK83 exhibited negligible Bsh activity compared to RK21 while no significant difference was observed among their acid and bile tolerance abilities ( $P > 0.05$ ).

## Immune status of WNIN mutant obese rats with reference to leptin and obesity

Two spontaneously mutated obese rat models viz., WNIN/Ob and WNIN/GR-Ob were developed from Wistar rats which are maintained by inbreeding at National Institute of Nutrition. Both these obese rat models are hyperinsulinemic, hypertriglyceridaemic, hypercholesterolemic and hyperleptinemic. However, both these strains differ in their glycemic index with WNIN/Ob being euglycemic and WNIN/GR-Ob exhibiting impaired glucose tolerance. These strains also develop kidney dysfunction and tumors as they cross one year of age and their life span is short (1 ½ year vs. 3 years of normal rats) suggesting impaired immune response.

Obese animals of both the strains showed altered cell mediated immune response in terms of reduced splenic CD4<sup>+</sup> helper T cells and CD3<sup>+</sup> Total T cells. Further, upon Hepatitis B vaccination, these obese animals exhibited reduced splenic lymphocyte proliferative response to HBsAg, low HBsAg specific IgG response (both WNIN/Ob and WNIN/GR-Ob obese) and altered macrophage function (only WNIN/GR-Ob obese). However, the factors involved in obesity associated immune dysfunction is still not clear.

In the present study, an attempt was made to study the effect of leptin on immune functions using WNIN/Ob and WNIN/GR-Ob strains. Leptin was administered intra peritoneally and its effect on the immune function in terms of splenic lymphocyte proliferative response to Con A by using tritiated thymidine incorporation method, splenic CD4/CD8 ratio by flow cytometry and Nitric oxide production by macrophages was studied. Further, we also studied whether the effect of leptin on immune function is mediated through its receptor and signaling molecules i.e., OBR and JAK2 protein expression.

## OBJECTIVE

To determine the role of leptin and leptin signaling on immune response in WNIN/Ob and WNIN/GR-Ob obese models.

## RESULTS

### 1. Body weight

Body weights were reduced upon starvation in both obese and lean groups, but no further difference in body weight was seen after leptin treatment (Table 4 & 5).

**Table 4. Changes in body weight with starvation in WNIN/GR-Ob**

Groups	Body weight (g)		change in body weight (g)
	Before starvation	After starvation	
<b>Obese</b>			
Starvation-leptin	464.3 ± 15.7 <sup>a</sup>	437.8 ± 6.83 <sup>b</sup>	26.5 ± 3.52 <sup>*</sup>
Starvation-PBS	490 ± 5.0 <sup>a</sup>	468.8 ± 7.0 <sup>b</sup>	21.2 ± 6.11 <sup>*</sup>
Free feeding	463.7 ± 4.02 <sup>a</sup>	466 ± 5.58 <sup>a</sup>	2.3 ± 0.00
<b>Lean</b>			
Starvation-leptin	257.3 ± 6.33 <sup>a</sup>	240.6 ± 8.66 <sup>b</sup>	16.7 ± 2.52 <sup>*</sup>
Starvation-PBS	250.3 ± 6.33 <sup>a</sup>	233.6 ± 4.66 <sup>b</sup>	16.7 ± 3.14 <sup>*</sup>
Free feeding	249.7 ± 7.97 <sup>a</sup>	247.5 ± 8.77 <sup>a</sup>	2.2 ± 0.57

Values are mean ± S.E; n = 8. Means that do not share common letter is significantly different at 0.05 level respectively. Comparisons were made between body weight changes before and after starvation (by students T-test). \*P < 0.05 by one-way ANOVA. Comparisons between starved PBS or leptin treated vs free feeding in obese or lean groups.

**Table 5. Changes in body weight with starvation in WNIN/Ob**

Groups	Body weight (g)		Change in body weight (g)
	Before starvation	After starvation	
<b>Obese</b>			
Starvation-leptin	416 ± 7.2 <sup>a</sup>	394.4 ± 10.9 <sup>b</sup>	21.6 ± 4.63 <sup>*</sup>
Starvation-PBS	430.5 ± 10.8 <sup>a</sup>	405.5 ± 10.6 <sup>b</sup>	25 ± 3.54 <sup>*</sup>
Free feeding	452 ± 17.4 <sup>a</sup>	452.5 ± 19.5 <sup>a</sup>	0.5 ± 0.0
<b>Lean</b>			
Starvation-leptin	244.5 ± 6.73 <sup>a</sup>	227.5 ± 3.22 <sup>b</sup>	17.0 ± 3.45 <sup>*</sup>
Starvation-PBS	233.8 ± 5.26 <sup>a</sup>	217.3 ± 4.80 <sup>b</sup>	16.5 ± 1.58 <sup>*</sup>
Free feeding	224.0 ± 6.66 <sup>a</sup>	223.5 ± 6.94 <sup>a</sup>	0.5 ± 0.0

Values are mean ± S.E; n = 8. Means that do not share common letter is significantly different at 0.05 level respectively. Comparisons were made between body weight changes before and after starvation (by students T-test). \*P < 0.05 by one-way ANOVA. Comparisons between starved PBS or leptin treated vs free feeding in obese or lean groups respectively.

## 2. Serum leptin levels

Leptin concentrations in sera were higher in both WNIN/Ob and WNIN/GR-Ob obese groups than in lean groups. In WNIN/GR-Ob, starvation significantly reduced serum leptin levels in lean animals only. Though, serum leptin levels were unaltered in lean animals, it was significantly reduced upon leptin treatment in obese animals (Table 6).

In WNIN/Ob, serum leptin levels were significantly reduced in both obese and lean groups upon starvation. Further, the serum leptin levels were unchanged upon leptin treatment (Table 7).

**Table 6. Serum leptin levels, CD4/CD8 ratio, Splenic lymphocyte proliferative response (T/C), LPS stimulated NO production by Peritoneal macrophages in WNIN/GR-Ob obese and lean animals**

Parameters	WNIN/GR-Ob Lean			WNIN/GR-Ob Obese		
	Free feeding	Starvation-PBS	Starvation-leptin	Free feeding	Starvation-PBS	Starvation-leptin
Serum leptin levels (ng/mL)	1.14±0.25 <sup>a</sup>	0.15±0.006 <sup>b</sup>	0.17±0.008 <sup>ab</sup>	37.2±0.96 <sup>c</sup>	35.2±1.1 <sup>c</sup>	25.0±2.04 <sup>d</sup>
CD4/CD8 ratio	2.01±0.01 <sup>a</sup>	1.74±0.01 <sup>b</sup>	2.07±0.07 <sup>ac</sup>	1.66±0.09 <sup>b</sup>	1.45±0.03 <sup>d</sup>	1.6±0.06 <sup>bd</sup>
Splenic lymphocyte proliferative response (T/C)	6.3±0.071 <sup>abc</sup>	3.37±0.38 <sup>ac</sup>	11.4±0.44 <sup>b</sup>	9.96±2.43 <sup>ab</sup>	6.09±1.59 <sup>abc</sup>	4.7±1.09 <sup>ac</sup>
LPS-stimulated NO production by Peritoneal macrophages (ng/ml)	3.2±1.2 <sup>a</sup>	4.3±0.71 <sup>a</sup>	7.1±0.18 <sup>b</sup>	6.8±0.2 <sup>b</sup>	4.8±1.6 <sup>ab</sup>	8.3±0.4 <sup>b</sup>

Values are mean±S.E; n= 8. Means that donot share common letter is significantly different at 0.05 level respectively (by one-way ANOVA). Comparisons were made between lean and obese phenotypes.

**Table 7. Serum leptin levels, CD4/CD8 ratio, Splenic lymphocyte proliferative response (T/C), LPS stimulated NO production by Peritoneal macrophages in WNIN/Ob obese and lean animals**

Parameters	WNIN/Ob Lean			WNIN/Ob Obese		
	Free feeding	Starvation-PBS	Starvation-leptin	Free feeding	Starvation-PBS	Starvation-leptin
Serum leptin levels (ng/mL)	0.29±0.08 <sup>a</sup>	0.12±0.00 <sup>b</sup>	0.17±0.003 <sup>ab</sup>	27±1.73 <sup>c</sup>	21±1.37 <sup>d</sup>	24.6±2.90 <sup>cd</sup>
CD4/CD8 ratio	2.08±0.06 <sup>a</sup>	1.83±0.04 <sup>b</sup>	2.13±0.06 <sup>ab</sup>	1.43±0.2 <sup>c</sup>	1.36±0.1 <sup>c</sup>	1.46±0.06 <sup>c</sup>
Splenic lymphocyte proliferative response (T/C)	9.62±0.981 <sup>a</sup>	7.9±1.73 <sup>a</sup>	9.9±2.31 <sup>a</sup>	15.7±4.8 <sup>a</sup>	10.73±1.83 <sup>a</sup>	10.6±1.44 <sup>a</sup>
LPS-stimulated NO production by Peritoneal macrophages (ng/ml)	9.1±1.7 <sup>ab</sup>	6.6±1.1 <sup>a</sup>	11±0.88 <sup>b</sup>	9.0±1.9 <sup>ab</sup>	9.9±1.48 <sup>ab</sup>	9.5±0.45 <sup>ab</sup>

Values are mean±S.E; n= 8. Means that don't share common letter is significantly different at 0.05 level respectively (by one-way ANOVA). Comparisons were made between lean and obese phenotypes

## 3. CD4/CD8 Ratio

In WNIN/GR-Ob, starvation significantly reduced the CD4/CD8 ratio in both obese and lean animals. However, leptin treatment increased CD4/CD8 ratio in lean animals only (Table 6).

In WNIN/Ob, starvation caused a significant reduction in CD4/CD8 ratio in lean animals only. Further, leptin treatment increased CD4/CD8 ratio in lean animals, but not in obese animals (Table 7).

**4. Splenic lymphocyte proliferative response to concavalin A**

In WNIN/GR-Ob, the lymphocyte proliferative response was unaltered upon starvation in both obese and lean animals. However, it was significantly increased upon leptin treatment in lean animals only (Table 6).

In both WNIN/Ob lean and obese groups, starvation did not alter the lymphocyte proliferative response to Con A. Further, leptin treatment also did not alter the lymphocyte proliferative response to Con A (Table 7).

**5. LPS stimulated peritoneal macrophage NO production**

In both WNIN/Ob and WNIN/GR-Ob, starvation did not alter NO production in both obese and lean animals. Further, leptin treatment did increase NO production in lean animals of both the strains.

**6. Immunoblotting**

It was not able to detect leptin-induced tyrosine phosphorylation of OBR and JAK 2 in splenic cells which may be due to insufficient sensitivity in the assays. In WNIN/GR-Ob animals, starvation did not alter OBR expression in lean animals, whereas it significantly increased OBR expression in obese animals. Further, leptin treatment had differential effects in both obese and lean animals. Thus, OBR expression was down regulated in obese animals and increased in lean animals by leptin treatment (Fig 6).

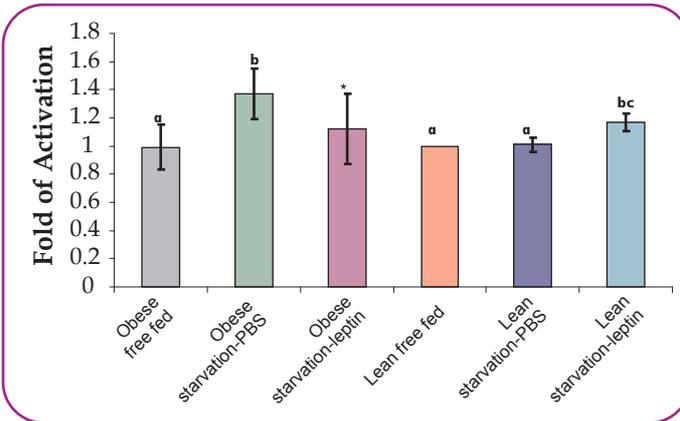
**Fig 6. OBR protein expression in WNIN/GR-Ob.**

**Fig. 6A**



120kDa 6A. Western blot of OBR protein in Obese free fed (Ofed), Obese starvation-PBS (OPBS), Obese starvation-leptin (OLEptin), Lean free fed (Lfed), Lean starvation-PBS (LPBS) and Lean starvation-Leptin (Lleptin).

**Fig. 6B**



6B. Quantification of OBR protein expression by densitometry. Means that don't share common letter is significantly different at 0.05 level respectively (by one-way ANOVA). \*p=0.06 Obese starvation-leptin and obese starvation-PBS.

In WNIN/Ob, OBR and JAK2 protein expression were comparable between obese and lean free fed animals. However, upon starvation in lean animals, though OBR expression was significantly reduced, JAK2 protein was unaltered. Further, leptin treatment though did not alter OBR expression, there was a significant trend towards increased JAK2 protein expression in lean animals (Fig 7 & 8). However, in obese animals, starvation did not alter OBR and JAK2 expression. Furthermore, leptin treatment also did not have any effect on OBR and JAK2 protein expression.

**Fig 7. OBR protein expression in WNIN/Ob**

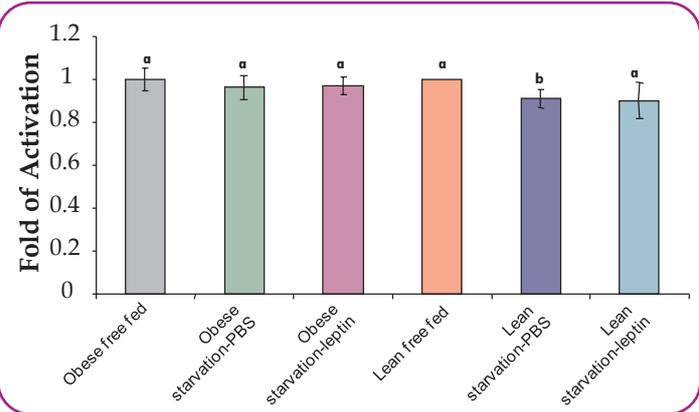
**Fig 7A**

OFED OPBS OLEPTIN LFED LPBS LLEPTIN



7A. Western blot of OBR protein in Obese free fed (Ofed), Obese starvation-PBS (OPBS), Obese starvation-leptin (OLEptin), Lean free fed (Lfed), Lean starvation-PBS (LPBS) and Lean starvation-Leptin (Lleptin).

**Fig 7B**



7B. Quantification of OBR protein expression by densitometry. Means that don't share common letter is significantly different at 0.05 level respectively (by one-way ANOVA).

**Fig 8. JAK2 protein expression in WNIN/Ob**

**Fig 8A**

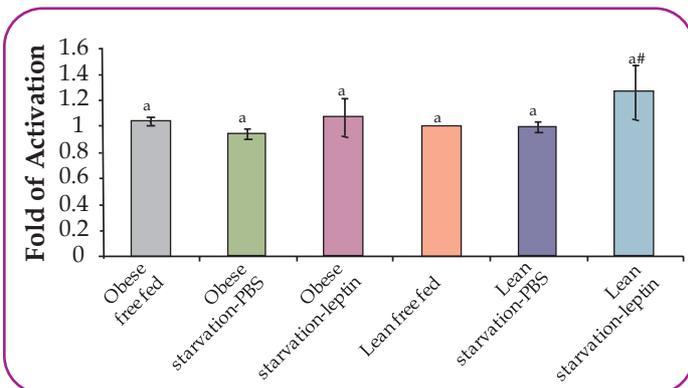
OFED OPBS OLEPTIN LFED LPBS LLEPTIN

125 kDa



8A. Western blot of JAK2 protein in Obese free fed (Ofed), Obese starvation-PBS (OPBS), Obese starvation-leptin (OLEptin), Lean free fed (Lfed), Lean starvation-PBS (LPBS) and Lean starvation-Leptin (Lleptin).

**Fig 8B**



8B. Quantification of OBR protein expression by densitometry. Means that don't share common letter is significantly different at 0.05 level respectively (by one-way ANOVA). #p=0.1 significant trend between lean starvation-leptin vs lean free fed and lean starvation-PBS.

**CONCLUSION**

In WNIN/Ob and WNIN/GR-Ob obese models there was reduced JAK2 and OBR (leptin receptor) expression respectively that might have contributed to leptin resistance, which was evident in the present study. Leptin resistance may contribute to impaired immune response observed in these models

Hepatitis B is an infection with serious long-term sequelae. Fortunately, a safe and effective vaccine against this infection is available. This vaccine has led to a major reduction in the prevalence of chronic HBV infection in several countries around the world.

Infant vaccination against hepatitis B began in India around 2003. The effect of the hepatitis B vaccine can be best assessed when the infected cohorts reach an age group of 5-7 years. It is thus now an opportune time to assess its impact on hepatitis B prevalence.

The current study will assess the prevalence of chronic hepatitis B among children who had received and who had not received hepatitis B immunization in infancy to assess the protective efficacy of hepatitis B vaccine in an Indian population.

This will help assess the effectiveness of hepatitis B immunization under field conditions in the Indian population. It may also provide data to help improve the delivery of hepatitis B immunization program.

A subunit vaccine containing the viral surface protein (hepatitis B virus surface antigen) is highly effective in preventing this infection. The use of this vaccine has been recommended among all newborns to prevent chronic HBV infection, and the disease burden associated with such infection. In countries where HBV infection is highly endemic, incorporation of hepatitis B vaccine in national childhood immunization program has been shown to lead to a marked reduction in prevalence of chronic HBV infection. In fact, introduction of hepatitis B immunization programs has been shown to be associated with a reduction in the rates of hepatocellular carcinoma among children.

In India, nearly 3-4% of healthy population has carrier state for HBV infection (intermediate endemicity: defined as HBV carrier rate in general population of 2%-8%). It has been estimated that this infection causes a significant disease burden. Hepatitis B vaccine has been included in childhood immunization program in selected areas in India over the last few years, as follows:

- a. Phase Ia (cities): This activity began in Hyderabad city in November 2002, and was expanded to 14 cities by December 2003. Till the year 2004, only children living in urban slums were covered. In 2005, the program was expanded to cover both slum and non-slum areas of these 14 cities.
- b. Phase Ib (districts): Between September and December 2003, hepatitis B vaccine was introduced as a pilot project in 33 districts across the country. Initially (in 2004), the program had a target of covering 80% of the eligible children. From 2005 onwards, this was increased to 100% of eligible children.
- c. Phase II: In the end of 2008, the administration of hepatitis B vaccine has been expanded to cover 11 states.

In addition, the state of Andhra Pradesh introduced hepatitis B vaccine in the entire state in 2003 on its own.

The dosage schedule used has been as follows:

- a. Initial: (a) 0, 6, 14 weeks (if birth dose can be given), or (b) 6, 10, 14 weeks.
- b. Current (since 2008): (a) 0, 6, 10, 14 weeks (if birth dose can be given), or (b) 6, 10, 14 weeks.

The impact of infant hepatitis B immunization programs can be best measured when the immunized cohorts reach the age of 5-7 years. This is because hepatitis B infections acquired till about 5 years of age have a high propensity to become chronic, whereas those acquired after this age rarely lead to chronic infection. Thus, majority of chronic hepatitis B infections that could occur have accumulated by this age.

The outcome measures used for assessing the effectiveness of hepatitis B vaccine in field study have been (i) HBsAg (total rate of chronic hepatitis B; vaccine is expected to lead to a reduction in this outcome measure), (ii) anti-HBc (total number of hepatitis B infections, irrespective of whether these lead to chronic hepatitis B infection or not), and (iii) anti-HBs (antibody titers; however these have a limitation in that these fall with time, and a person may be protected even though antibody levels may fall below detection limit).

The Government of India is interested in assessing the impact of hepatitis B vaccination in the areas covered under the pilot project. The current proposal is a step in that direction.

## **AIMS AND OBJECTIVES**

- ❖? To determine the prevalence rate of HBsAg among Indian children who received hepatitis B immunization during infancy, in comparison with children who were not immunized during infancy.
- ❖? To determine the prevalence rate of total anti-HBc among Indian children who have received hepatitis B immunization during infancy as part of the national immunization program, in comparison with children who were not immunized during infancy.
- ❖? To assess the prevalence and level of anti-HBs antibodies among Indian children who have received hepatitis B immunization during infancy, in comparison with children who were not immunized during infancy.

## **MATERIALS AND METHODS**

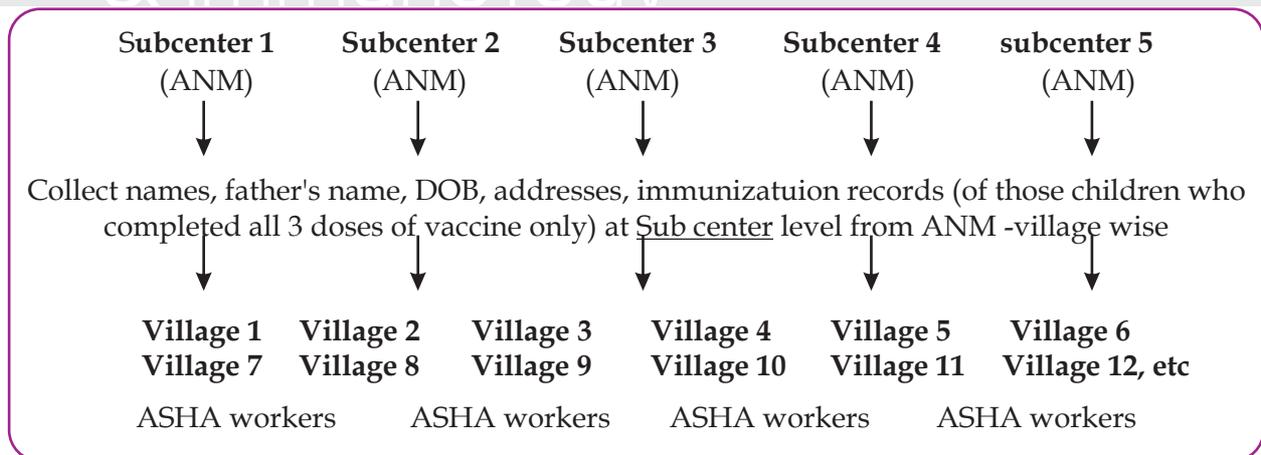
As per the original plan of work, after a preliminary survey, five districts of Andhra Pradesh (Rangareddy, Medak, Nizamabad, Karimnagar and Nalgonda) were selected keeping in mind logistic consideration (specimen processing and transport), and availability of vaccination data. In each district thus selected, two mandals or PHCs were identified by cluster sampling technique. Permission was sought from the state health authorities and from District Medical and Health Officers (DMHOs) of the selected districts. Thereafter, each Mandal and PHCs located at each mandal headquarters were visited and Medical Officers, ANMs, CHOs, Health Visitors and Health Supervisors were requested to prepare a list of children who had been vaccinated during the period November 2003 to December 2004. The lists contained the child's name, parents' name, address, date of birth, and date of vaccination of those children who have received all 3 doses of hepatitis B vaccine. Each ANM, with the help of ASHA workers and Anganwadi workers (AWW) who are based in villages within the subcenter, were able to provide us data of all the villages she was responsible for.

From each study subject, 3 ml of blood specimen was drawn and separated serum samples were frozen. HBsAg, anti-HBc (total) and anti-HBs quantified with ELISA kits. Out of 5100 blood samples, 2700 were vaccinated and 2400 were unvaccinated.

### **Plan of work for sample collection at Village level**

#### **PHC**

Meet with medical officer, ANM's of all subcentres and enlist their help to collect data regarding the villages they cover.



Each ASHA worker/ANM should help in collecting all the children in her village to one place, in the village itself, on a pre-decided day where their blood samples can be drawn after rechecking the records.

Attrition was to the extent of 5% and was due to mental retardation (1%), acute illness (2%) at the time of recruitment, migration (2%) or due to unknown vaccination status (Table 8).

## RESULTS

Data analysis is going on.

**Table 8**

	Total number	Anti- HBs	Anti - Hbc	HBs- Ag
Positive among Vaccinated	2674	1415	28	4
Positive among unvaccinated	2350	417	42	4
% positive		52.92	1.05	0.15
% positive		17.74	1.79	0.17
Chi-squared (vaccsunvacc)		667.90	4.99	
p value (vaccsunvacc)		<0.0000001	0.01	NS

Percent prevalence of Hepatitis B surface antigen was less than 1%. Hepatitis B vaccine does not seem to offer protection from HBS-Ag prevalence.

## 4 Role of probiotics on growth and morbidity in children

Specific probiotic strains have been shown to reduce the incidence or shorten the duration of diarrhoea. Some strains have also been shown to reduce the risk for disease. One of the main targets of probiotics is the intestine, but health benefits are not limited to that site. Selected probiotics have, among others, been shown to improve immune response providing a mechanism for health benefits not directly associated with the intestine. The intestine is the body's largest immune organ and changes in the composition and/or activity of the intestinal microflora may be, in part, responsible for an observed change in immune response.

*L. paracasei* Lpc-37 or *B. lactis* Bi-07 are highly tolerant to acid and bile, have strong adhesion property to intestinal cell lines, thereby well suited for intestinal survival and function. *B. lactis* Bi-07 has been shown to exert a moderate but significant protection from the intestinal inflammation model, demonstrating this strain's ability to interact with and beneficially balance the intestinal mucosal immune response. *L. paracasei* Lpc-37 may have an influence on immune regulation, as demonstrated through induction of IL-12 *in vitro*. The study tries to demonstrate their role on acute diarrhoea and immune response in humans. In this context the study has great significance. The probiotics *L. paracasei* Lpc-37 or *B. lactis* Bi-07 used in the study were expected to provide a health benefit, if present. Furthermore, the dose used would be close to what is technically feasible in a fermented dairy product.

The study aimed to investigate the influence of selected probiotics (*L. paracasei* Lpc-37 or *B. lactis* Bi-07) on the incidence and duration of acute diarrhoea, as well as immune function, nutritional status and changes in intestinal microflora. Diarrhoeal morbidity (duration and episodes -1 year) was collected by 7 day recall method. Use of antibiotics and history of febrile episodes were also collected. Stool microflora (by PCR) and Immune markers- Calprotectin and Secretory IgA - were done by ELISA. Diarrhoeal pathogens were investigated in children with diarrhoea. Growth was assessed by height, weight and skin fold measurements.

## AIMS AND OBJECTIVES

To study the effect of *L. paracasei* (Lpc-37) or *B. lactis* (Bi-07) supplementation for 6 months period, on diarrhoeal morbidity (duration and episodes), febrile episodes, immune function, nutritional status and gut flora in apparently normal children.

### Work done during the year

The children who were recruited in June 2010 and randomized into three groups were continued with supplementation for a total period of 9 months, i.e till May 2011. Monthly measurements of weights were carried out till July 2011 and every 4<sup>th</sup> monthly anthropometry including skin fold measurements were done, the last in July 2011. A final measurement of heights and weights at the 16<sup>th</sup> month of supplementation was also done in December 2011. Stool samples were collected after six months and again after 9 months of supplementation for immune markers & microbiota analysis and aliquoted. A portion of these aliquots were transported to Danisco Oy, Kantvik, Finland. Blood samples were also collected after supplementation for 9 months and biochemical analysis of serum samples for micronutrients has been initiated. Analysis for Secretory IgA (sIgA) and Stool Calprotectin by ELISA has been done for baseline, 6<sup>th</sup> month followup samples and a sub sample of 9<sup>th</sup> month follow up samples. Data entry and analysis is underway. Dropouts: From 7<sup>th</sup> month to 9<sup>th</sup> month there were 13 dropouts, due to migration. During the first 6 months of supplementation, there were 34 dropouts, which were either due to parent's unwillingness to continue with supplementation or migration. However, no adverse effects were observed during the entire course of the supplementation period or thereafter.

## RESULTS

The Mean Age, weight and height of children in all the three groups were comparable (Table 9). The male-to-female ratio of subjects recruited in each group was also comparable. Roughly 12 % of the children were undernourished and similar proportion was stunted. Nutritional status of all the three groups was comparable between males and females of different groups. Undernourishment (BMI for age) was comparable between the three supplemented groups at baseline and end line, in both males and females. All the children in the 3 groups showed consistent weight gain and linear growth (Fig. 9). The increment of weight and height was comparable between the groups. Percent body fat and Lean body mass was comparable between the groups and there was a decrement in percent body fat and a simultaneous increase in Lean

body mass in all the groups over a period of 1 year (Table 10). This is the first study showing that probiotic supplementation for 9 months period will not adversely impact growth in children. Diarrhea and respiratory infections were prevalent in 3 and 10% respectively, and parasitic infestation was prevalent in 10% of the children (Table 9). The proportion of children with diarrhea was comparable between groups at baseline. When we compared the prevalence of diarrhea for July month before supplementation of probiotics and July month after supplementation there was substantial reduction in diarrhea in children treated with *L. paracasei* (Lpc-37); in contrast there was no change in the prevalence of diarrhea in children treated with *B. lactis* (Bi-07) or placebo (Table 11). However, there was no change in the prevalence of respiratory tract infections.

**Table 9. Demographic data of children in the study**

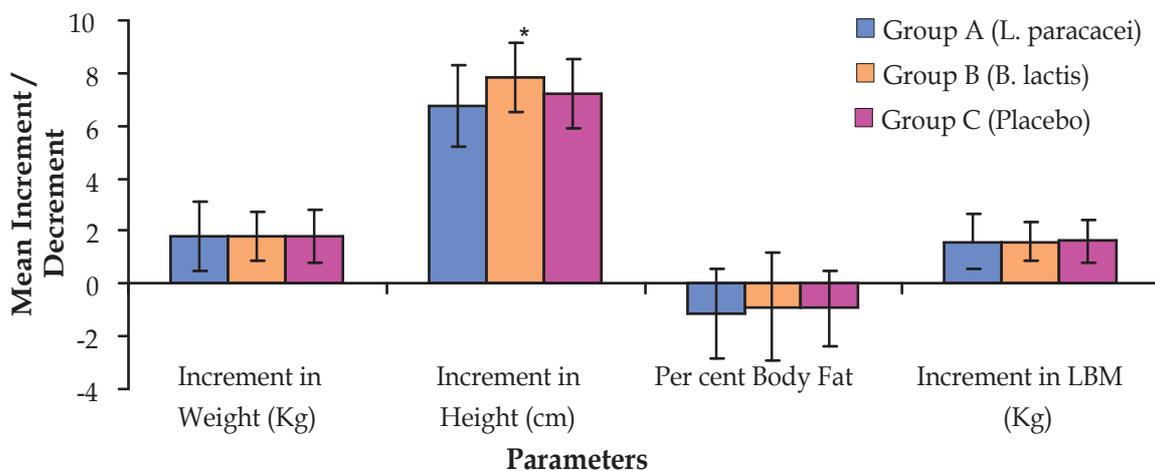
Baseline parameters	Group A ( <i>L. paracasei</i> )	Group B ( <i>B. lactis</i> )	Group C (Placebo)	Whole Group (Pooled)
Age (months)	37.5 ± 8.21	38.9 ± 9.24	37.4 ± 8.79	37.9 ± 8.76
Weight(kg)	12 ± 1.8	11.5 ± 1.86	11.6 ± 1.74	11.7 ± 1.81
Height (cm)	90.7 ± 5.7	88.5 ± 8.21	89.3 ± 6.27	89.5 ± 6.88
Parasitic infestations	9.6 (125) <sup>a</sup>	13.1 (130) <sup>a,b</sup>	5.6 (124) <sup>a,c</sup>	(379)

Values of age and height are Mean ± SD.

Values for Parasitic infestations are proportions. Total number of subjects in parenthesis.

<sup>a,b</sup> Comparison between baseline morbidity of 3 groups in each row

**Fig 9. Comparison of anthropometry parameters between supplemented groups**



**Table 10. Increment in anthropometry parameters between supplemented groups**

Parameters	Group A ( <i>L. paracasei</i> ) (94)	Group B ( <i>B. lactis</i> ) (100)	Group C (Placebo) (103)
Increment in Weight (Kg)	1.78 ± 1.31 <sup>a</sup>	1.79 ± 0.91 <sup>a</sup>	1.81 ± 0.98 <sup>a</sup>
Increment in Height (cm)	6.75 ± 1.51 <sup>a</sup>	7.83 ± 1.31 <sup>b</sup>	7.24 ± 1.31 <sup>a</sup>
Per cent Body Fat	-1.14 ± 1.67 <sup>a</sup>	-0.87 ± 2.05 <sup>a</sup>	-0.93 ± 1.42 <sup>a</sup>
Increment in LBM (Kg)	1.6 ± 1.04 <sup>a</sup>	1.59 ± 0.73 <sup>a</sup>	1.62 ± 0.82 <sup>a</sup>

Values are mean ± SE. Total number of subjects in parenthesis.

Values with similar super scripts between the groups do not differ significantly

**Table 11. Comparison of Diarrhoea, and CED in the study groups at different time periods**

Morbidity		Group A ( <i>L. paracasei</i> )		Group B ( <i>B. lactis</i> )		Group C (Placebo)	
		July 2010 (Baseline)	July 2011 (End line)	July 2010 (Baseline)	July 2011 (End line)	July 2010 (Baseline)	July 2011 (End line)
Diarrhoea		3.3 (120) <sup>a</sup>	0.0 (105) <sup>α</sup>	3.4 (119) <sup>a</sup>	2.7 (113) <sup>α</sup>	2.5 (122) <sup>a</sup>	2.9 (104) <sup>α</sup>
Under nourishment	Males	5.4(56) <sup>a</sup>	2.3(44) <sup>α</sup>	3.2(63) <sup>a</sup>	2.1(47) <sup>α</sup>	1.8(60) <sup>a</sup>	7.5(40) <sup>α</sup>
(BMI for age) (<3SD)	Females	1.7(61) <sup>a</sup>	2.4(41) <sup>α</sup>	7.7(52) <sup>a</sup>	7.0(43) <sup>α</sup>	1.6(64) <sup>a</sup>	2.1(48) <sup>α</sup>

Values are proportions. Total number of subjects in parenthesis.

<sup>a,b,c</sup> Comparison between baseline morbidity of 3 groups in each row

<sup>α,β</sup> Comparison between endline morbidity of 3 groups in each row

<sup>#</sup> Comparison between baseline and endline morbidity within the groups

Values with similar super scripts between the same time points of different groups and between different time points within the group do not differ significantly.

Mc-nemar test performed (as they are dependent samples), CED: Chronic energy deficiency

## CONCLUSION

*L. paracasei* (Lpc-37) induced significant reduction in diarrhea; in contrast there was no change in the prevalence of diarrhea in children treated with *B. lactis* (Bi-07). Both the probiotics did not have any effect on respiratory infections. Weight gain and linear growth was normal in children supplemented with *L. paracasei* Lpc-37 and *B. lactis* Bi-07.

## V. BASIC STUDIES

### 1 Folic acid, vitamin B<sub>12</sub> status and its association with leptin and anthropometric indices of adiposity among urban adolescent boys belonging to low and middle income group, Hyderabad, India

Relatively little information is available on the influence of deficiencies of folic acid and vitamin B<sub>12</sub> on changes in body composition. In addition, the type of deficiency which could have specific effects on the body composition is also unknown. We considered adolescent population ideal to understand the effects of folic acid and vitamin B<sub>12</sub> deficiency due to the lack of interventions related to both nutrients and the dramatic body composition changes during adolescence in addition to the increased demands during growth. Therefore, the objectives of the study were to assess the status of folic acid and vitamin B<sub>12</sub> in a group of adolescent boys belonging to low and middle income group and to elucidate the relationship between status of these nutrients with anthropometric measures of body composition, leptin and lipid profile and the changes that may occur in a span of 12 months.

#### MATERIALS AND METHODS

The results reported are part of a prospective study on 'Stress, allostatic load and micronutrients status among students' carried out in the academic year 2009-2010. The study was carried out in five Government single-gender Boys' schools belonging to the Greater Hyderabad Municipal Corporation, Hyderabad, Andhra Pradesh, India. The protocols and procedures has approved by institutional ethics committee. Approval was also sought from the Board of Intermediate studies, Government of Andhra Pradesh. A written informed assent was obtained from the participants and consent from their parents for inclusion in the study.

Apparently healthy students, 15-19 y without any diagnosed hormonal abnormalities, congenital anomalies and those who are not currently under medication or not taking any multivitamin or mineral tablets for the past 1 year were selected. The data presented here includes 145 observations; a sub-sample of 380 that were required for the larger study. The longitudinal data represents 80 paired measurements for blood parameters and 77 for anthropometry after one year. A short nutrition education programme for improving micronutrient status was carried out for three months and measurements were taken after a gap of one month.

Following a probability- proportional to size of the student- strength of higher secondary level, eligible students were selected randomly for blood sampling at baseline. The blood sample (10mL) was collected on 12 hour fasting between 8.00-9.00 am in each school and aliquoted into 0.5ml for analysis of hemoglobin and remaining collected into heparinized vacutainers, processed and stored at -20°C till analysis.

#### STUDY VARIABLES

##### *Anthropometry*

All anthropometric measurements were done by trained investigators. Body weight of the participants was taken without shoes and heavy clothing, using a calibrated SECA electronic weighing scale (Seca, Hanover, MD), with a precision of 100 g. A portable anthropometric rod (Galaxy Scientifics, India) was used for measuring height, to the nearest of 0.1 cm, using standard procedures.

The height for age Z score (HAZ) cut-off used for stunting was less than -2 Z scores. Underweight, overweight and obesity was defined as less than -2, 1.04-1.65 and >1.65 BMI- for-

age-Z scores (BAZ) for children below 19 years. For eight participants who were 19-19.9 years, an adult cut-off of body mass index (BMI)  $25\text{kg}/\text{m}^2$  was considered as overweight.

The biceps, triceps, sub-scapular and suprailiac skinfold thicknesses were measured on the non-dominant side of the body using Harpenden skinfold calipers (CMS Instruments, London, UK) to the nearest 0.2 mm. The measurements were repeated thrice and an average was used employing standard protocol by manufacturers. The investigator carrying out the measurements was trained by a qualified physical anthropologist. Reliability statistics was calculated using 12 pair of measurements collected on three different days between the anthropologist and the investigator. The overall intra class correlation coefficient of the measurements was 0.998 and the correlation of the 4 site measures ranged from 0.95- 0.99.

The sum of all four skin-fold thickness measurements was used to calculate body density by the formula of Durnin & Womersley. For age group less than 17 years, the formula by Durnin and Rahaman was employed. Both the equations have been validated in Asian Indians. The body fat was calculated using Siri's equation. The difference between body weight and body fat was taken as fat free mass (FFM). Waist and hip circumferences were measured using a fibre reinforced tape to the nearest 0.1cm using standard protocol.

Household possession of assets was collected as proxy variables for calculation of standard of living index.

### *Biochemical variables*

Leptin was analyzed in plasma by ELISA kit with an intraassay variation of  $<3.3\%$ , interassay variation of  $<5.4\%$  and assay sensitivity of  $7.8\text{ pg}/\text{mL}$  (R&D systems Inc. Minneapolis, USA). Folic acid and vitamin  $\text{B}_{12}$  was analysed using dual count solid phase no boil assay with an interassay and intrassay variation of  $<10\%$  and analytical sensitivity of  $34\text{ pg}/\text{mL}$  and  $0.3\text{ ng}/\text{mL}$  for vitamin  $\text{B}_{12}$  and folic acid, respectively (Siemens medical solutions diagnostics, Los Angeles, USA). The cut-off used to define folic acid deficiency was  $<4\text{ ng}/\text{mL}$  and that of vitamin  $\text{B}_{12}$  it was  $<203\text{ pg}/\text{mL}$ . Total cholesterol and HDL cholesterol was assayed using the kits as per manufacturer's instructions (Biosystems, Barcelona, Spain).

### **Statistics**

The relationship between folic acid, vitamin  $\text{B}_{12}$  and anthropometric indices of adiposity was tested using the baseline data (N=145). The endline measurements were only used to test the changes in anthropometric measures and micronutrient status over a period of one year. The height for age Z scores (HAZ) and BMI for age Z scores (BAZ) were calculated using the WHO Anthroplus software. In the absence of data on Tanner stages of development we intended to use the increment in height as a proxy for development. For this, an arbitrary cut-off of 2cm was used which was close to the median increment of 1.85 cm in height of the participants.

Data analysis was carried out using SPSS 19.0 (SPSS, Inc. USA). Descriptive statistics was used to summarize results. Pearson correlation and Spearman's rho correlation was used to assess the relationship between variables. Paired t test and the Wilcoxon sign rank test were used for comparison of change in variables depending upon the spread of the data. Independent t test and ANOVA was employed for group comparisons when data was normally distributed and Kruskal-Wallis H followed by Mann Whitney U when data was skewed. For such skewed data a P value of  $<0.017$  was considered significant and otherwise a P value  $<0.05$  was considered as significant difference for multiple comparisons. Log transformation was done for leptin due to large variation.

Adjustment for age and standard of living index was done using ANCOVA. A stepwise regression model was tested with body weight as a dependent variable and age, economic status, hemoglobin, fat %, folic acid, vitamin  $\text{B}_{12}$  and leptin as predictors. A similar model was tested with

fat% as dependent variable with the inclusion of body weight instead of fat% in the list of predictors mentioned above.

## RESULTS

### Baseline characteristics

The mean age of the students was  $16.6 \pm 1.24$  years, height  $163.6 \pm 6.96$  cm and weight  $47.6 \pm 7.40$  kg. Stunting (23.4%) and underweight (34.5%) were prevalent among the participants. The mean values for folic acid were  $5.4 \pm 1.81$  ng/mL, for vitamin B<sub>12</sub> it was  $250 \pm 112.5$  pg/mL. The percentage fat was  $13.9 \pm 4.1$  with a mean fat mass of  $6.7 \pm 2.81$  kg (Table 12).

**Table 12. Characteristics of the study population at baseline**

Variables	Mean	SD
Age (y)	16.6	1.24
Height (cm)	163.6	6.96
Body weight (kg)	47.6	7.40
BMI(Weight kg/ Height m <sup>2</sup> )	17.7	2.24
Waist- Hip Ratio	0.81	0.04
Fat percent*	13.9	4.1
Fat mass (kg)	6.7	2.81
Fat free mass (kg)	40.8	5.61
Hemoglobin (g/dL)	14.1	1.67
Plasma Folate (ng/mL)	5.4	1.81
Plasma Vitamin B <sub>12</sub> (pg/mL)	250	112.5
Leptin (ng/mL)†	0.91	0.54-1.49
Total Cholesterol (mg/dL)	119	23.5
HDL (mg/dL)	32.8	5.62
<b>Prevalence of malnutrition (%)</b>		
Stunting based on score	23.4	
Undernutrition based on BAZ score	34.5	
Overweight	1.4	

\* Fat percentage is calculated by using Siri's equation: Fat (%) = [(495/bodydensity) - 450].

Body density Y is derived from  $Y = 1.1533 - 0.0643x$  for children <16years and  $Y = 1.1620 - 0.0630x$  for children 17-19 years, where x= log (sum of 4 skinfolds).

† presented as median and inter-quartile range.

### Relationship between folic acid, vitamin B<sub>12</sub>, anthropometry and blood chemistry

Vitamin B<sub>12</sub> deficiency was 39% while folic acid deficiency was 17%.

An age independent significant decrease in HDL cholesterol (P=0.010), increase in body weight (P=0.012), BMI (P=0.032) and fat free mass (P=0.043), waist circumference (P=0.006), subscapular skinfold thickness (P=0.019) was observed in the folic acid deficient group. Vitamin B<sub>12</sub> deficient group showed a significant increase (P=0.020) in body fat percentage and fat mass (P=0.037) compared to sufficient group after controlling for age and economic status. Folate status was significantly higher in the vitamin B<sub>12</sub> deficient group (Table 13).

In the stepwise regression model, plasma folate status was found to be a significant predictor of body weight ( $\beta = -0.639$ , P=0.020, R<sup>2</sup> = 42.7%) after controlling for the potential confounders. Vitamin B12 status was found to be a significant predictor of fat percentage after controlling for confounders ( $\beta = -0.0047$ , P=0.033, R<sup>2</sup> = 49.3%).

**Table 13. Relationship between folic acid, vitamin B12, lipid profile, leptin and anthropometry at baseline**

Variables	Folic acid Mean ± SD ng/mL			Vitamin B <sub>12</sub> Mean ± SD pg/mL		
	<4 (N= 25)	>4 (N=120)	P value	<203 (N=57)	>203 (N=88)	P value
Age (y)	16.9 ± 1.4	16.6 ± 1.1	0.175	16.6 ± 1.04	16.7 ± 1.27	0.552
Height (cm)	165.0 ± 5.53	163.4 ± 7.21	0.294	162.6 ± 6.89	164.3 ± 6.97	0.158
Weight (kg)	51.0 ± 8.14	46.9 ± 7.07	0.019	48.1 ± 8.9	47.2 ± 6.31	0.481
BMI	18.7 ± 2.46	17.5 ± 2.16	0.032	18.1 ± 2.56	17.5 ± 2.02	0.215
Fat %	14.7 ± 3.87	13.7 ± 4.13	0.235	14.9 ± 4.07	13.2 ± 3.98	0.020
Fat mass (kg)	7.7 ± 3.11	6.5 ± 2.72	0.060	7.4 ± 3.14	6.3 ± 2.52	0.037
FFM (kg)	43.3 ± 5.78	40.3 ± 5.46	0.043	40.7 ± 6.64	40.9 ± 4.87	0.828
WC (cm)	67.1 ± 5.49	63.9 ± 5.29	0.006	65.3 ± 6.63	64.0 ± 4.50	0.353
Waist-hip ratio	0.82 ± 0.05	0.81 ± 0.05	0.199	0.82 ± 0.04	0.81 ± 0.05	0.355
TC (mg/dL)	116.4 ± 26.15	119.8 ± 22.95	0.505	119.9 ± 21.65	118.9 ± 24.63	0.808
HDL- C (mg/dL)	30.3 ± 5.27	33.49 ± 5.56	0.010	32.2 ± 5.86	33.4 ± 5.44	0.189
Vitamin B12 (pg/mL)	271.2 ± 107.9	245.2 ± 113.46	0.295	-----	-----	-----
Folic acid (ng/mL)	-----	-----	---	5.8 ± 1.79	5.1 ± 1.77	0.036
Leptin (ng/mL)	1.7 ± 2.23	1.3 ± 1.36	0.163	1.57 ± 1.133	1.18 ± 0.99	0.716

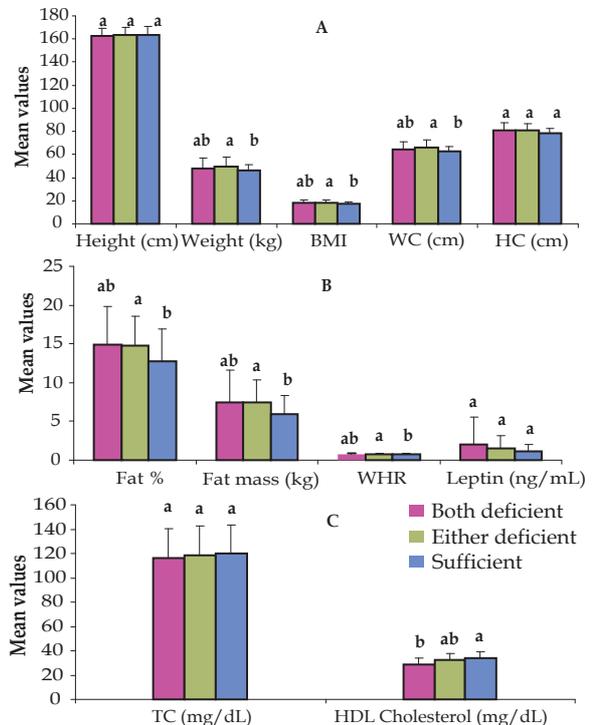
BMI, Body mass Index; FFM, Fat free mass; WC, waist circumference; TC, total cholesterol; HDL-C, HDL Cholesterol. The values are expressed as mean and SD. Comparisons are made between deficient and sufficient groups. The age and economic status adjusted P values <0.05 were considered significant. The relationship between vitamin B12 and fat % was additionally tested for HAZ scores.

Further, categorization was done as sufficient in both, deficient in both and deficient in either of the two micronutrients to assess the effect of combined deficiency and single nutrient deficiency of either folic acid or vitamin B<sub>12</sub> on adiposity. A greater body weight, BMI, fat %, fat mass and waist circumference, WHR was observed in the group with either folate or vitamin B<sub>12</sub> deficiency. The group with both deficiencies also showed similar pattern but was not statistically significant except for a lower HDL cholesterol. No difference was observed in height, leptin or total cholesterol in any of the groups (Fig 10).

**Longitudinal changes in parameters**

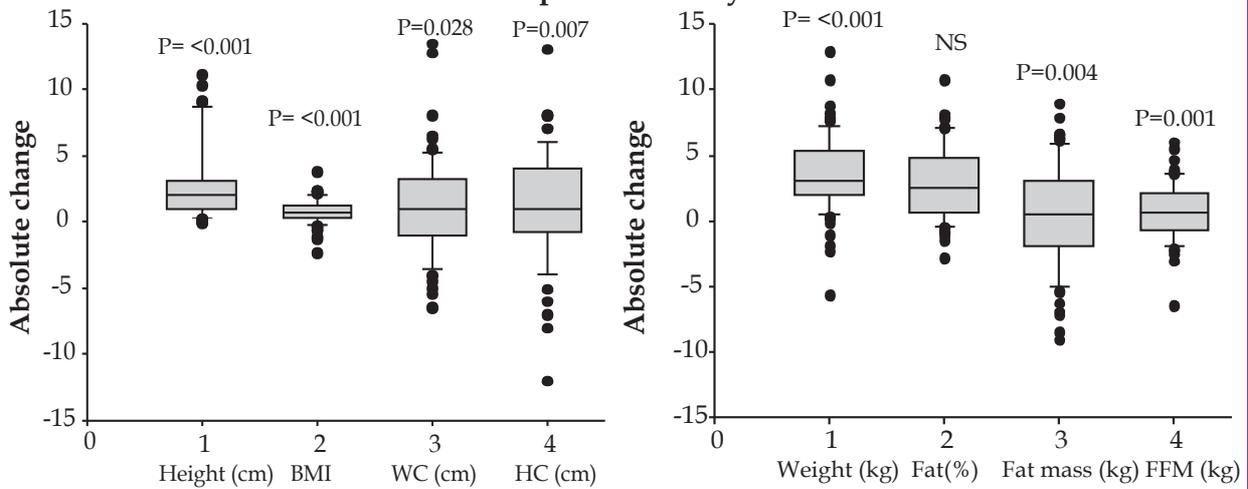
The deficiency of vitamin B<sub>12</sub> increased from 39% to 66.2% and the deficiency of folic acid marginally increased to 20.8% over 12 months. There was a significant increment in total cholesterol (119.7±24.40 to 158.0 ± 21.71, P<0.001) and HDL cholesterol (33.0 ± 5.33 to 42.94 ± 8.46, P<0.001). There was a wide variation observed in changes in anthropo-metric parameters (Fig 11). The changes in fat % and leptin (data not shown) were not significant. Increment in body weight, height, BMI, fat mass, FFM, waist and hip circumferences were significant (P<0.001).

**Fig 10. Mean difference in parameters based on single/dual deficiency & sufficiency of folic acid and vitamin B12 at baseline**



Comparisons were made using one way ANOVA with posthoc Bonferroni correction. Wherever the variables were not normally distributed, the non-parametric test was applied. The bars are shown as Mean + SD and the bars with different superscripts are considered significant at P<0.05.

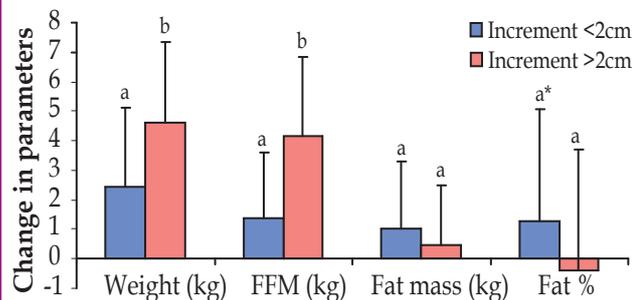
**Fig 11. Box plot showing variation in the changes in anthropometric variables over a period of one year.**



N=77, Boxes represent the middle 50<sup>th</sup> percentile and vertical lines extend to the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Median values are marked by a line within each box. Outliers, defined as values <10<sup>th</sup> percentile and >90<sup>th</sup> percentile, are identified as solid circles. The changes were tested using paired t test. BMI, body mass index; WC, waist circumference; HC, hip circumference.

The mean height increment in participants who gained  $\geq 2$  cm height was  $4.6 \pm 2.80$  cm and those who gained  $<2$ cm was  $0.9 \pm 0.69$  cm. About 47.5% students gained  $\geq 2$  cm height. Body weight and fat free mass ( $P < 0.001$ ) increased significantly in the group which had height increment  $\geq 2$  cm while the group with  $<2$ cm increment in height tended to have a higher positive change in fat % ( $-0.38 \pm 4.1, 1.3 \pm 3.81$  respectively for  $\geq 2$ cm and  $<2$  cm height gain category),  $P = 0.074$  (Fig. 12). Leptin and fat % ( $R = 0.327, 0.621, P < 0.001$ ) as well as fat mass ( $R = 0.375, 0.716, P < 0.001$ ) showed a positive correlation in both the groups. There was a negative trend between vitamin B<sub>12</sub> and fat % ( $r = -0.195$ ) in  $<2$ cm height increment group (data not shown).

**Fig 12. Changes in parameters in subjects based on increment in height**



Comparison was made between participants with height increment  $<2.0$ cm (N=38) and  $>2.0$  cm (N=39). Bars are mean  $\pm$  SD and bars with different superscripts are significantly different ( $P < 0.05$ ). \* $P = 0.074$  on Mann Whitney U test. FFM, fat free mass; NS, not-significant.

**CONCLUSIONS**

- ✎ The deficiency of folic acid and vitamin B<sub>12</sub> was prevalent among the adolescents from low and middle income group.
- ✎ The deficiencies appeared to influence body composition particularly folic acid influencing body weight, and vitamin B<sub>12</sub> preferentially influencing body fat %.
- ✎ Leptin showed a positive relationship with body fat but showed no significant relationship with the two nutrients.
- ✎ Large scale epidemiological studies are required to further elucidate the magnitude of the problem and designing of interventions.

The two major factors responsible for high prevalence of iron deficiency anemia are poor dietary density and low bioavailability of iron from cereal-pulse based vegetarian diet. Application of principles of dietary diversification can provide evidence for practicing this as the strategy to improve iron bioavailability. However, there is a paucity of information on iron availability from individual foods and their combinations to facilitate dietary diversification to improve iron bioavailability. Measuring iron dialyzability is used as surrogate of iron bioavailability. The existing colorimetric method is not sensitive to detect low level of iron present in the dialysate especially from plant sources. In the present project attempt has been made to increase throughput and sensitivity of method by use of radioisotope and fluorescent probe Phen GreenSK.

### METHODOLOGY

The method using 6 well plate setup was modified for the estimation of dialyzable iron using  $^{59}\text{Fe}$  and PGSK. Briefly, the samples were subjected to *in vitro* gastric and intestinal digestion with/without ascorbic acid and transferred to the 6 well culture plates. Samples were incubated for 2 h at 37 °C with constant shaking. At the end of incubation, a transwell insert-ring with a dialysis membrane (MWCO: 6-8000) was placed on top of each well of the plate in such a way that the membrane was in the contact with the contents in the well and the inserts were filled with 2 mL of PIPES buffer (pH 6.3) and incubated for further 2 h at 37 °C. At the end of the incubation, dialysate from the upper chamber was collected.

The  $^{59}\text{Fe}$  radioactivity in the dialysate 0.5 ml counted in liquid scintillation analyzer and the cpm converted to dpm considering 50% counting efficiency of  $^{59}\text{Fe}$ . Iron estimation by colorimetry was done in 0.2 ml dialysate using bathophenanthroline in a 96 well microtiter plate. For iron estimation using PGSK, 20  $\mu\text{l}$  of aliquot and 100  $\mu\text{l}$  of 5  $\mu\text{M}$  PGSK in imidiazole-ascorbate buffer (pH 7.2) was taken along with iron standard ranging from 10 -150 picomoles/ well in 96-well plate for fluorescent measurements. Fluorescence was read at excitation 485, emission 528 after 5' at 25° C in a multi-mode detection microplate reader.

#### *Comparisons of the methods*

Experiments were done with various food samples (rice, wheat & pea) and  $\text{FeCl}_3$  in the presence and absence of ascorbic acid, a potent enhancer of iron absorption as described above. Samples in each well were spiked with 80 nCi radioactive iron. Dialyzable iron was estimated from the same dialysate using the three methods that is (i) colorimetry, (ii) radioisotope and (iii) PGSK.

### STATISTICAL ANALYSIS

The mean and standard deviation were computed using Microsoft Excel. Statistical analysis of the data was performed using SPSS version 11.0 package. Paired "t" test and Pearson's correlation was computed for comparing the methods. The results were considered significant if p value was < 0.05. Band-Altman plots were generated from the data to compare the methods using SPSS version 19.0.

### RESULTS

#### *Standardization of PGSK method*

Conditions for estimation of iron using PGSK were standardized with 20  $\mu\text{l}$  of aliquot of iron standard ranging from 10 -150 picomoles/ well or sample and 100  $\mu\text{l}$  of 5  $\mu\text{M}$  PGSK in imidiazole-ascorbate buffer (pH 7.2). The measurement of fluorescence were done in a 96-well

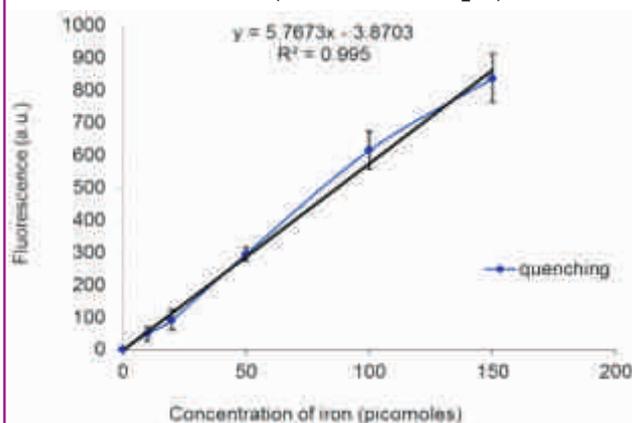
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plate at excitation 485, emission 528 after 5' at 25° C. Fluorescence quenching was found to be linear in the concentration range 10 -150 p moles/ well (Fig 13).

Dialyzability of iron from samples in the presence and absence of iron absorption enhancer (ascorbic acid) was studied and compared with other two methods. The assay characteristics of all the methods are given in Tables 14 and 15. All the three methods were comparable ( $p > 0.05$ ) and showed very good linear relationship ( $r > 0.96$ ). The sensitivity of fluorescence probe and radioisotope method for iron were 13.6 p moles and 21.7 p moles, respectively compared to the sensitivity of 1.27 n moles with the colorimetry.

Band-Altman plots indicated that the values obtained using PGSK is very close to isotope and 95% of the points were well within the limits of agreement (Fig 14).

**Fig 13. Phen Green SK fluorescent Probe method (Standard Graph)**



Iron induced quenching of PGSK. Values shown represent mean  $\pm$  SD ( $n=3$ ). PG SK at 5  $\mu$ M concentrations was incubated with increasing concentration of iron for 5' and fluorescence emission was read at 528  $\pm$  20 nm at 25° C.

**Table 14. Comparison of assay characteristics: colorimetric, radioisotope and Phen Green SK method**

Characteristics	Colorimetry	Isotope	PGSK
Sensitivity	1.27 n moles	21.7 p moles	13.6 p moles
Variability	8.4 %	15.7 %	9.78 %
Advantages	Inexpensive	High specificity Less cumbersome High throughput	Less time Smaller volume High throughput
Disadvantages	Matrix effects Requires additional steps Less sensitivity External contamination	Handling of radioisotope Hazardous chemical High variability External contamination	External contamination

**Table 15 - Dializability of iron from food sources in the presence and absence of ascorbic acid by colorimetry, radioisotope and Phen Green SK method**

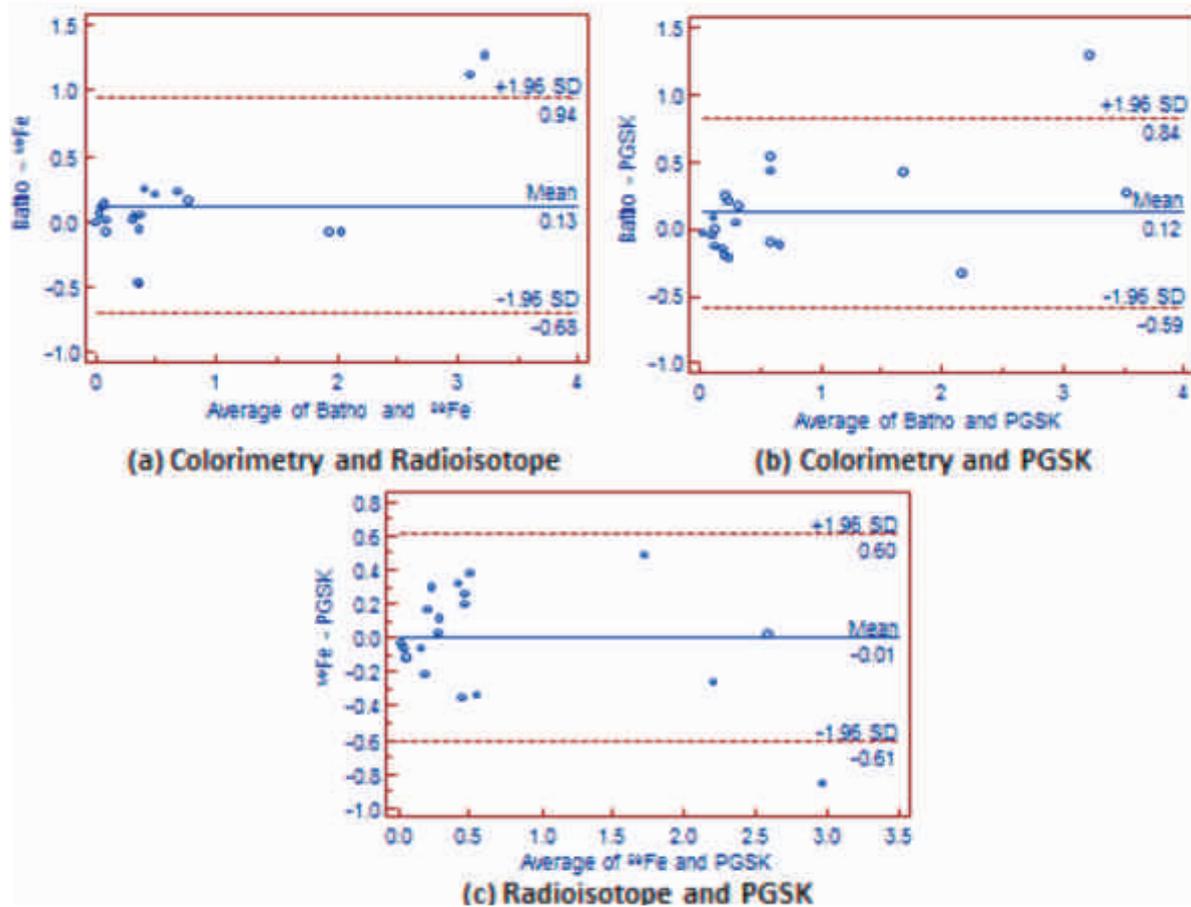
Methods	r value*	p values**('t' test)
Colorimetry Vs. Radioisotope	0.96	0.366
Colorimetry Vs. PGSK	0.98	0.239
PGSK Vs. Radioisotope	0.97	0.949

\*Pearson's correlation coefficient, \*\*Based on paired in 't' test,  $\alpha=0.05$ ,  $n=10$  pairs

### Advantages:

The PGSK is a rapid method as it does not involve additional steps of protein precipitation, centrifugation, require very small amount of sample (20  $\mu$ l), lesser incubation time and well suited for assay in 12 well plates.

Fig 14. Comparisons between different assay types: Bland-Altman Plot



Experiment was done with 10 different samples. Experiment was done in triplicate and replicated. Each data point in the plot represents an average value (triplicates clubbed to generate single value) for single experiment.

### Conclusion

A very sensitive high throughput rapid screening tool for assessing iron dialyzability from foods low in iron content was developed. These methods can be used for screening and selecting most promising diversified foods for iron bioavailability.

## 3 Developmental origins of adiposity and insulin resistance: Role of peri/postnatal manganese status and high fat feeding in later life

Foetal growth is largely a reflection of nutrient and oxygen supply to the foetus. Hence, prenatal nutrition is the most important programming stimulus. The 'Barker Hypothesis' also known as the 'Foetal Origins Theory', suggests that unfavourable foetal environment causes nutrients to be diverted, leading to programming of a number of systems that influence health in later life. Developmental programming influences body composition through appetite regulation, epigenetic modification of key regulatory genes, altered fat deposition and adipocyte metabolism. A better understanding of the relationship of birth size to maternal nutrition is critical for planning

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effective intervention to improve birth weight in Indian babies. Dietary intake of energy and proteins of rural Indian mothers is low and so is the consumption of foods rich in micronutrients (eg, dairy products, meat, fresh fruits and green leafy vegetables).

Multiple micronutrient deficiencies during pregnancy and/or lactation which are associated with LBW and increased perinatal mortality/morbidity are common in the developing world, where the prevalence of low birth weight is around 30% while that of adult onset degenerative diseases is reaching epidemic proportions. Considering their importance in modulating metabolism, micronutrient status (vitamins and minerals) in foetal and early life (in addition to the pre-natal micronutrient status of the mother) may increase the risk of adult onset degenerative diseases in the offspring.

Previous studies showed that, maternal multivitamin and multimineral deficiencies lead to increased body adiposity and altered lipid/ glucose metabolism in WNIN rat offspring. It was also reported subsequently, that maternal deficiency of trace elements : Cr, Mg and Zn produce similar effects in the offspring which were not reversible by rehabilitation. Manganese, an essential trace element activates numerous enzyme systems involved in glucose metabolism, energy production and is an activator/major constituent of several metalloenzymes, hormones and proteins of humans. In experimental animals, Mn deficiency leads to diabetic-like glucose intolerance wherein glucose utilization is impaired.

Keeping in view i) the role of Mn in carbohydrate and lipid metabolisms, ii) the increase in consumption of fat rich foods in the present population and iii) the role of Mn in foetal programming for adult diseases is not yet known. The present studies were carried out to validate/negate the following hypothesis.

## **HYPOTHESIS**

Maternal Mn restriction *per se* alters body composition (adiposity), impairs glucose/insulin metabolism in the rat offspring and also makes them more susceptible to such insults by high fat feeding in later life.

## **OBJECTIVES**

1. To assess the effect of maternal and/or peri/postnatal Mn deficiency on the development of adiposity and insulin resistance in the offspring.
2. To assess the effects if any, of rehabilitation of Mn restricted mothers and/or offspring, from different time points of initiation and duration on the development of adiposity, IR as well as changes in the related physical/physiological parameters in the offspring.
3. If the body adiposity and/or IR status are altered in the offspring, study the associated biochemical mechanisms in the target tissues like skeletal muscle and adipose tissue.
4. Assess the differences if any among the control, Mn restricted and rehabilitated offspring in their susceptibility to the ill effects of postnatal high fat feeding.

## **Experimental procedures**

The animal experiment was carried out in accordance with the 'principles of laboratory animal care' with the approval of IAEC which ensures that the NIH guidelines are strictly followed. About thirty female weanling Wistar NIN (WNIN) rats were divided into two groups. The first group containing 24 rats were fed a basal diet (AIN-93G, with 18% casein as the source of protein) containing 0.33 mg Manganese/kg diet for 16 weeks (Mn restricted group, MnR). The other group of 6 rats received the same diet as above but it contained 8.92 mg Manganese/kg diet (Control group, MnC). This accounted for 96 % restriction of Mn in the deficient animals. The animals were fed *ad libitum* for 16 weeks and they had free access to de-ionised water. Their daily food intake and

weekly body weight gain were monitored. The blood Mn levels were monitored at the end of the feeding regime. The animals were mated with control males (2 females: 1 male) and some animals from the restricted group were rehabilitated with control diet from conception (MnRC) while the remaining animals continued on restricted diet. Body weight of the rats before mating and their weight gain during pregnancy were recorded. At parturition, six mothers from the restricted group were rehabilitated to control diet (MnRP) and the remaining mothers continued on restricted diet until weaning. Litter size was adjusted to seven on postnatal day 3 in mothers of all groups. Half the number of restricted offspring were put on control diet (MnRW) from weaning while the remaining continued on restricted diet throughout (MnR). Appropriate control animals (n=6) were maintained (on control diet) throughout the study. The effects of Mn restriction *per se* (effects on WNIN female rats) and maternal Mn restriction in modulating body composition, lipid metabolism, glucose tolerance and insulin secretion in the offspring were studied. Both male and female offspring were taken for the study.

### **Effect of Mn restriction *per se* on glucose tolerance, insulin resistance and reproductive performance of WNIN female rats**

As expected, there was a significant difference in the blood Mn levels between the control and restricted animals. Despite creating ~ 96 % Mn deficiency, the daily food intake and body weight gain were comparable between the groups and this was in line with earlier reports of similar nature. There were no changes in the glucose levels or the insulin status of the MnR rats nor did the animals show any significant changes in the physiological as well as biochemical parameters monitored. Contrary to the previous findings of low birth weight offspring in maternal Mg and Zn restriction, maternal Mn restriction did not influence weight gain during pregnancy, neonatal mortalities, litter size at birth or birth weight.

### **Foetal programming for adiposity and IR: Role of Mn status**

Chronic maternal Mn restriction showed only transient changes in body fat % (as assessed by TOBEC) both in male and female offspring and was not associated with any changes in visceral adiposity (adiposity index). In contrast to the earlier findings in the offspring of Mg and Cr restricted rat dams these results suggest that maternal Mn restriction may affect the body composition of the offspring albeit transiently. However, despite no change in visceral adiposity, there was a marked increase in the circulating levels of some pro-inflammatory cytokines both in male and female MnR offspring and the changes were at best correctable partially. Perplexingly, however no such changes in adipocytokines profile was seen in the adipose tissue. In line with the transient increase in body fat % seen in male and female offspring, changes in lipid profile were also transient in nature. Taken together these findings suggest that, maternal Mn restriction may affect only the adipocyte function but not its development in the WNIN rat offspring.

Transient change in body fat % was associated with a decrease in LBM% and FFM% in male (at 3 months) and female (at 6 months) MnR offspring, suggesting that maternal Mn restriction transiently decreases muscle and/or bone mass in the offspring. But there was no change in the tissue associated fat % suggesting that maternal Mn restriction may not play any significant or persistent role in fat distribution in the body. That Oil Red 'O' staining of the liver in male offspring was comparable between the MnC and MnR offspring probably corroborates the above inference. Despite no persistent effect on FFM %, insulin stimulated glucose uptake by muscle was significantly lower in MnR offspring which probably suggesting that maternal Mn restriction may modulate muscle function. However, lack of any difference in the expression (protein levels) of insulin signaling molecules such as Akt, IRS-1 and IR- between the two groups does not seem to support this finding. It is therefore looks possible that maternal Mn restriction could impair the activation (phosphorylation) of these molecules and/or translocation of Glut 4 to membrane, which was however not studied.

Maternal Mn restriction induced fasting hypoinsulinemia in male offspring albeit at 6 months of age only and rehabilitation did not correct the insult. These observations are in line with previous report that Mn deficiency decreased insulinogenesis. These observations probably suggest that maternal Mn restriction during gestation and its post natal continuation could be important in regulating insulin secretion in offspring and this was also corroborated by the transient decrease in insulin AUC observed during OGTT. That there were no consistent effects in fasting glucose may be explained at least to some extent by the observation that activities of most of the gluconeogenic enzymes studied (which play a major role in maintaining fasting glucose levels) were comparable among the groups of offspring. On the other hand lack of any persistent effect of maternal Mn restriction on oral glucose tolerance in the offspring could be due to the lack of any consistent effect of maternal Mn restriction on adipose and muscle mass (the two tissues important in clearing circulating glucose post prandially) in offspring, notwithstanding the effects it appeared to have on insulinogenesis and insulin sensitivity of the muscle.

In contrast to the previous observations of increased oxidative stress in the micronutrient restricted offspring, there were no changes in the oxidative stress as well as corticoid stress in the offspring, possibly suggesting that they may not be associated with maternal Mn-induced transient changes in the offspring and probably hinting as to why the changes observed were only transient in nature.

#### **Effect of high fat feeding in the later life of control and manganese restricted offspring**

Considering that the effects of maternal Mn restriction on various parameters monitored in the offspring (discussed above) were transient and inconsistent in nature. Whether these changes are precipitated on superimposing with an additional nutritional insult such as high fat feeding in their later life (from 9 months of age) which was equivalent to young adolescent age of humans were assessed.

As expected, offspring fed on high fat diet for 8 weeks gained more weight (23%) than those fed the standard-fat diet (control group). That MnR offspring fed HF diet gained significantly higher body weight than MnR fed normal fat (NF) diet appears to suggest maternal Mn restriction may predispose the offspring to adverse effects of nutritional insult in their later life. That male HF MnR offspring had higher body fat % than MnR at 12 and 15 months support the studies showing an association between increased fat intake and obesity development. That these changes were not seen at 18 months of age probably suggest the transient nature of the changes. That LBM% and FFM% of HF MnR male offspring were lower than those of MnR offspring was in line with previous studies from our lab showing decreased LBM% and FFM% in offspring having increased body fat %.

Observation of increased TNF- $\alpha$  levels in the plasma of HF MnC and HF MnR animals than their corresponding controls was in line with literature that activity of the adipose tissue derived adipocytokine was modulated by high fat feeding. That such increase in TNF- $\alpha$  levels were also seen in the NF fed MnR rats suggests that chronic dietary high fat feeding predisposes MnR rats to increased pro-inflammatory status. The observation of increased PAI-1 levels in adipose tissue of HF MnR than MnR rats was in line with the increased visceral adiposity seen in them and supports the literature that PAI-1 levels are strongly associated with visceral adiposity. In contrast to the reports that chronic high fat feeding in rats reduces whole body glucose disposal rate and impairs skeletal muscle glucose metabolism, no effect on the basal as well as insulin stimulated glucose uptake were observed by the muscle in the HF MnR offspring, suggesting that high fat feeding may not increase the susceptibility of these offspring to altered muscle function.

High dietary fat intake impairs glucose tolerance and decreases insulin sensitivity in both humans and animals. The finding that AUC glucose was higher at 18 and AUC insulin at 15 and 18

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months of age in HFMnR male rats compared to MnR seems to strongly support the above literature. Significantly, higher AUC insulin in HFMnR than MnR (at 15 months) female offspring also supports the same. Indeed, this study has for the first time shown that chronic high fat feeding increases the susceptibility of MnR rats to altered glucose tolerance in later life.

### **Effect of rehabilitation on high fat feeding**

Maternal Mn restriction predisposed the offspring to high fat feeding induced changes in body composition, carbohydrate and lipid metabolisms. Therefore, the effects of Mn rehabilitation on high fat feeding induced alterations in the offspring was assessed. HF feeding induced increase in body weight was higher in MnR offspring rehabilitated from parturition and weaning probably suggests the importance of Mn during lactation and later in life in modulating body fat % was observed. Indeed it was surprising to see no effect of HF feeding on visceral adiposity of the rehabilitated offspring probably suggesting differential susceptibilities of body fat content and distribution to the effects of high fat feeding vis a vis Mn restriction.

The lack of effects of HF feeding on LBM% and FFM% of rehabilitated animals seems to suggest that muscle development and physiology was perhaps programmed before birth and thus postnatal HF feeding may not affect it. There was no difference in the peripheral fat deposition in the animals as evidenced by the comparable levels of TAF% between the various groups of rats further confirming the observations on muscle development and physiology. That there was no effect of HF feeding on muscle glucose uptake in the rehabilitated groups suggests that rehabilitation prior to HF feeding could have prevented the changes in the muscle function.

Increase in glucose levels in HFMnR compared to corresponding rats fed normal fat diet was in line with literature that long term high fat feeding produces a wider variation in fasting plasma glucose levels with some hyperglycemia. The observation that all rehabilitated groups of rats had significantly higher activity of Pyruvate carboxylase in liver was also in line with studies which show that high fat feeding increases gluconeogenesis.

This study demonstrated that, maternal Mn restriction predisposes the offspring to the adverse effects of high fat feeding in later life viz, increased central adiposity, fat deposition in liver and induction of a pro-inflammatory state and possibly leading to a metabolic syndrome like situation.

### **CONCLUSIONS**

- ❖ Maternal Mn restriction transiently altered the body composition of male and female rat offspring.
- ❖ It modulated adipocyte function (induction of a proinflammatory state) but not its development.
- ❖ Maternal Mn restriction played an important role in muscle function but not its development.

This study has for the first time demonstrated that maternal Mn restriction predisposes the offspring to increased central adiposity, fat deposition in liver, induction of a pro-inflammatory state and altered glucose tolerance specially when fed high fat diets, possibly leading to a metabolic syndrome like situation.

## 4 Effect of different methods of cooking on phenolic content and antioxidant activities of pulses and legumes commonly consumed in India

Excess production of free radicals and lipid peroxides underlie the pathogenesis of degenerative diseases like atherosclerosis, carcinogenesis, diabetes, cataract and ageing etc. Epidemiological evidence suggests that diet plays a crucial role in prevention of the non-communicable degenerative diseases. Plant derived antioxidants, such as flavonoids and related phenolic compounds are reported to have multiple biological effects, including antioxidant activity.

Although, more than 7000 phytochemicals have been identified in plant foods, a large percentage remains to be identified. Phytochemicals present in plant foods exert health beneficial effects, as they combat oxidative stress in the body by maintaining a balance between oxidants and antioxidants. Pulses and legumes are one of the major foods in developing countries like India. Data on cooking losses of nutrients and other health beneficial effects of food grains is scanty. Considering the known loss of micronutrients on cooking, a study was conducted whether similar losses occur in the phenolic content and their antioxidant activities on different types of domestic cooking are common in India.

### MATERIALS AND METHODS

Eleven types of pulses and legumes commonly consumed in India (based on the NNMB survey) were collected from three market outlets of Hyderabad. Samples were analyzed in duplicates and mean values are presented on fresh weight basis. Total quantity of each sample collected was between 200-250g and the edible portion of the sample was used for evaluating the effects of domestic cooking. Each sample was divided into four parts (25 grams each). First portion was processed as such to know its natural (raw) antioxidant activity, while the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> portions of the sample were subjected to conventional, pressure and microwave methods of cooking respectively. Briefly, 25 grams of food sample was cooked in 100 ml of water for 6 - 15 minutes (in case of conventional cooking it took about 15 minutes, pressure cooking was done in a domestic pressure cooker under standard conditions (about 10-12 minutes) whereas micro wave cooking was of 6 minutes duration. Cooking vessel was normally covered with a lid while cooking (exception: conventional cooking). To estimate antioxidant activity in raw (unprocessed) food, the 1<sup>st</sup> portion of 25g sample was ground in domestic blender and extracted with 80% methanol containing 0.1% HCl. A similar extraction procedure was followed for the food grains subjected to different types of domestic cooking and final volumes of all extracts were made equal with 80% methanol. Standard extraction and estimation protocols described earlier (Annual reports 2006-2011) were adopted. While the soluble total phenolic content (TPC) was determined by the Folin-Ciocalteu colorimetric method, the anti-oxidant activity (AOA) was determined by two different methods. 1. FRAP (Ferric Reducing/ Scavenging) 2. DPPH radical scavenging activity.

A total of eleven commonly consumed legumes/ pulses were chosen to study the effect of domestic cooking on phenolic and antioxidant activities (Table 16-18).

### RESULTS

1. Among the pulses and legumes (raw, unprocessed), total phenolic content was the highest in whole green gram (264mg/100g) followed by black rajmah (146mg/100g). Interestingly, green gram dhal had the least phenolic content (41 mg/100g). The difference in phenolic content of green gram whole and dhal thus appears to be due to the husk, known to contribute high phenolic contents in grains.

**Table 16. Effect of domestic processing on total Polyphenol content of commonly consumed Indian pulses and legumes**

Sl. No.	Common Name	Botanical Name	Phenolic Content (mg/100g Gallic acid Eq)				p Value
			Raw	Conventional	Pressure	Microwave	
1	Bengal gram dhal	<i>Cier arietinum</i>	92.6 <sup>a</sup> (100)	90.6 <sup>a</sup> (98)	98.6 <sup>a</sup> (106)	86.0 <sup>a</sup> (93)	NS
2	Bengal gram dhal (roasted)	<i>Cier arietinum</i>	116.3 <sup>a</sup> (100)	105.6 <sup>a</sup> (91)	108.6 <sup>a</sup> (93)	102.0 <sup>a</sup> (88)	NS
3	Bengal gram (whole grains)	<i>Cier arietinum</i>	114.0 <sup>a</sup> (100)	154.6 <sup>b</sup> (136)	176.3 <sup>c</sup> (154)	113.3 <sup>d</sup> (99)	0.024
4	Black gram dhal (with out peel)	<i>Phaseolus mungo Roxb</i>	69.3 <sup>a</sup> (100)	58.6 <sup>b</sup> (85)	60.0 <sup>b</sup> (86)	51.3 <sup>c</sup> (74)	0.022
5	Green gram dhal	<i>Phaseolous aureus Roxb</i>	41.3 <sup>a</sup> (100)	43.6 <sup>a</sup> (106)	43.0 <sup>a</sup> (104)	34.0 <sup>c</sup> (82)	NS
6	Green gram dhal (whole)	<i>Phaseolous aureus Roxb</i>	284.3 <sup>a</sup> (100)	249.3 <sup>b</sup> (88)	269.3 <sup>c</sup> (95)	243.6 <sup>b</sup> (86)	0.019
7	Lentil	<i>Lens esculenta</i>	64.3 <sup>a</sup> (100)	64.6 <sup>a</sup> (100)	59.0 <sup>a</sup> (92)	56.0 <sup>a</sup> (87)	NS
8	Peas green(dry)	<i>Pisum sativum</i>	82.3 <sup>a</sup> (100)	84.0 <sup>a</sup> (102)	103.3 <sup>b</sup> (126)	75.6 <sup>c</sup> (92)	0.024
9	Red gram dhal (without peel)	<i>Cajanus cajan</i>	70.0 <sup>a</sup> (100)	83.6 <sup>b</sup> (119)	81.6 <sup>b</sup> (117)	74.0 <sup>a</sup> (106)	0.035
10	Rajmah (Black)	<i>Phaseolus Vulgaris</i>	146.6 <sup>a</sup> (100)	186.0 <sup>b</sup> (127)	195.6 <sup>c</sup> (133)	159.3 <sup>c</sup> (109)	0.020
11	Soya been	<i>Glycine maxmerr</i>	81.6 <sup>a</sup> (100)	82.0 <sup>a</sup> (100)	98.3 <sup>a</sup> (121)	94.3 <sup>a</sup> (116)	NS

Mean values (n=3) were compared by Non-parametric Kruskal Walies H test of one way ANOVA. Values in a row with different superscripts are significantly different at p < 0.05. Percent gain or loss calculated with the raw value taken as 100%. Percent recovery values are given in parenthesis.

- DPPH scavenging activity of rajmah (black) was highest (160 mg/100g) followed by whole green gram dhal (113 mg/100g) whereas DPPH scavenging activity was the least in green gram dhal (21mg/100g).
- FRAP activity was the highest in black rajmah followed by soya been and the lowest was in green gram dhal. The FRAP values were 6852, 3778 and 1066 mg/100g respectively (Table 18) in these three foods.
- Effect of different cooking methods on antioxidant activity (AOA) of each food grain was compared with the antioxidant activity and phenolic contents of the unprocessed sample. Over all, different cooking methods did not show any consistent cooking losses, but mixed trend of increase and/ or decrease were observed (Table 16-18).
- Effect of cooking on TPC is presented in Table-16. Nine out of 11 legumes samples studied showed a maximum of 20% increase or decrease in their TPC during different types of cooking. Interestingly however, during conventional and pressure cooking, whole Bengal gram and rajmah showed 27 and 54% increase respectively in their TPC.

**Table 17. Effect of domestic processing on DPPH activity of commonly consumed Indian pulses and legumes**

Sl. No	Common Name	Botanical Name	DPPH (mg/100g Trolox Eq)				p Value
			Raw	Conventional	Pressure	Microwave	
1	Bengal gram dhal	<i>Cier arietinum</i>	42.6 <sup>a</sup> (100)	43.3 <sup>a</sup> (102)	43.6 <sup>a</sup> (102)	40.0 <sup>a</sup> (94)	NS
2	Bengal gram dhal (roasted)	<i>Cier arietinum</i>	31.3 <sup>a</sup> (100)	34.3 <sup>a</sup> (110)	31.3 <sup>a</sup> (100)	25.6 <sup>a</sup> (82)	NS
3	Bengal gram (whole grains)	<i>Cier arietinum</i>	68.6 <sup>a</sup> (100)	100.0 <sup>b</sup> (146)	95.3 <sup>b</sup> (139)	60.3 <sup>c</sup> (88)	0.022
4	Black gram dhal (with out peel)	<i>Phaseolus mungo Roxb</i>	35.0 <sup>a</sup> (100)	29.0 <sup>a</sup> (83)	30.0 <sup>a</sup> (86)	24.6 <sup>a</sup> (70)	NS
5	Green gram dhal	<i>Phaseous aureus Roxb</i>	21.3 <sup>a</sup> (100)	19.3 <sup>a</sup> (91)	17.6 <sup>a</sup> (83)	18.6 <sup>a</sup> (87)	NS
6	Green gram dhal (whole)	<i>Phaseolous aureus Roxb</i>	113.6 <sup>a</sup> (100)	184.3 <sup>b</sup> (162)	159.3 <sup>c</sup> (140)	171.3 <sup>bc</sup> (151)	0.027
7	Lentil	<i>Lens esculenta</i>	35.6 <sup>a</sup> (100)	38.0 <sup>a</sup> (107)	35.3 <sup>a</sup> (99)	36.6 <sup>a</sup> (103)	NS
8	Peas green(dry)	<i>Pisum sativum</i>	51.0 <sup>a</sup> (100)	55.3 <sup>a</sup> (108)	56.0 <sup>a</sup> (110)	42.0 <sup>b</sup> (82)	0.040
9	Red gram dhal (without peel)	<i>Cajanus cajan</i>	42.0 <sup>a</sup> (100)	49.3 <sup>a</sup> (117)	56.3 <sup>a</sup> (134)	42.0 <sup>a</sup> (100)	NS
10	Rajmah (Black)	<i>Phaseolus Vulgaris</i>	160.0 <sup>a</sup> (100)	182.3 <sup>a</sup> (114)	170.3 <sup>a</sup> (106)	174.0 <sup>a</sup> (109)	NS
11	Soya been	<i>Glycine maxmerr</i>	75.6 <sup>a</sup> (100)	61.3 <sup>b</sup> (81)	59.3 <sup>c</sup> (78)	71.6 <sup>a</sup> (95)	0.023

Mean values (n=3) were compared by Non-parametric Kruskal Wallies H test of one way ANOVA. Values in a row with different superscripts are significantly different at p<0.05. Percent gain or loss calculated with raw value taken as 100%. Percent recovery values are given in parenthesis.

- DPPH scavenging activity in legumes cooked by different cooking methods also showed a mixed/ inconsistent trend (Table 18). Nine out of eleven legumes studied showed less than 20% increase or decrease of cooking losses. It was however interesting that whole green gram showed a the highest increase in DPPH activity in all cooking methods studied, with the increase ranging from 40-62% as compared to its DPPH content in the unprocessed form.
- Different types of domestic cooking also showed inconsistent and varied effects on FRAP in different legumes (Table 18). Nine out of eleven legumes studied showed less than 20% variation in FRAP values. While whole green gram and dry green peas showed higher increase in FRAP ranging from 41-102% in different methods of cooking, lentil and red gram dhal showed 34 and 73% increase albeit during pressure cooking only.
- It was however of interest that overall the percent increase or decreases found vis a vis their content in unprocessed food showed similar trend in different cooking methods in a given legume.

- Studies from other parts of the world showed similar mixed trends on the effect of cooking on antioxidant parameters of potatoes and vegetables. Similar mixed trends were observed in the previous study in green leafy vegetables (Annual report 2010-11). The observed increase during cooking could be due to the liberation of high amounts of antioxidant compounds due to thermal destruction of cell wall and sub cellular compartments.

**Table 18. Effect of domestic processing on FRAP activity of commonly consumed Indian pulses and legumes**

Sl. No	Common Name	Botanical Name	FRAP (mg/100g FeSO <sub>4</sub> Eq)				p Value
			Raw	Conventional	Pressure	Microwave	
1	Bengal gram dhal	<i>Cier arietinum</i>	1679 <sup>a</sup> (100)	1909 <sup>a</sup> (114)	1968 <sup>a</sup> (117)	1973 <sup>a</sup> (118)	NS
2	Bengal gram dhal (roasted)	<i>Cier arietinum</i>	1466 <sup>a</sup> (100)	1711 <sup>a</sup> (117)	1359 <sup>a</sup> (93)	1367 <sup>a</sup> (93)	NS
3	Bengal gram (whole grains)	<i>Cier arietinum</i>	2283 <sup>a</sup> (100)	2560 <sup>b</sup> (112)	2676 <sup>b</sup> (117)	2177 <sup>a</sup> (95)	0.033
4	Black gram dhal (with out peel)	<i>Phaseolus mungo Roxb</i>	1515 <sup>a</sup> (100)	1420 <sup>a</sup> (94)	1470 <sup>a</sup> (97)	1265 <sup>a</sup> (83)	NS
5	Green gram dhal	<i>Phaseous aureus Roxb</i>	1066 <sup>a</sup> (100)	1371 <sup>a</sup> (128)	1042 <sup>a</sup> (98)	938 <sup>a</sup> (88)	NS
6	Green gram dhal (whole)	<i>Phaseolous aureus Roxb</i>	3098 <sup>a</sup> (100)	5490 <sup>b</sup> (177)	5785 <sup>c</sup> (187)	5505 <sup>b</sup> (178)	0.025
7	Lentil	<i>Lens esculenta</i>	1534 <sup>a</sup> (100)	1652 <sup>a</sup> (108)	2058 <sup>a</sup> (134)	1625 <sup>a</sup> (105)	NS
8	Peas green(dry)	<i>Pisum sativum</i>	1846 <sup>a</sup> (100)	3027 <sup>a</sup> (164)	3734 <sup>b</sup> (202)	2609 <sup>c</sup> (141)	0.016
9	Red gram dhal (without peel)	<i>Cajanus cajan</i>	2446 <sup>a</sup> (100)	3133 <sup>b</sup> (128)	4251 <sup>c</sup> (173)	2646 <sup>b</sup> (108)	0.016
10	Rajmah (Black)	<i>Phaseolus Vulgaris</i>	6852 <sup>a</sup> (100)	6809 <sup>a</sup> (99)	7171 <sup>b</sup> (105)	7915 <sup>c</sup> (115)	0.025
11	Soya been	<i>Glycine maxmerr</i>	3778 <sup>a</sup> (100)	3504 <sup>a</sup> (93)	3714 <sup>a</sup> (98)	3502 <sup>a</sup> (93)	NS

Mean values (n=3) were compared by Non-parametric Kruskal Wallies H test of one way ANOVA. Values in a row with different superscripts are significantly different at p<0.05. Percent gain or loss calculated with raw value taken as 100%. Percent recovery values are given in parenthesis.

**Table 19. Rank Correlation between Phenolic content vs. DPPH and FRAP in different cooking methods of Pulses and Legumes**

TPC vs AOA	Raw	Traditional	Pressure	Microwave	Homogeneity
TPC vs DPPH	0.689	0.801	0.793	0.780	? <sup>2</sup> = 1.23 , p= 0.746
TPC vs FRAP	0.573	0.701	0.619	0.706	? <sup>2</sup> = 1.12 , p= 0.772
DPPH vs FRAP	0.918	0.909	0.895	0.916	? <sup>2</sup> = 0.31 , p= 0.959

All correlations are significant at p<0.01 (n=11) and correlations are similar across the methods. Between the methods all the parameters are significantly correlated (TPC Vs DPPH; TPC Vs FRAP & DPPH Vs FRAP).

Alternately it could be due to thermal destruction and/ or production of stronger radical-scavenging antioxidants due to thermal/ chemical reactions.

11. Correlation among the TPC and the two AOA parameters were determined in the legumes in the unprocessed as well as during the three different types of domestic cooking. For this purpose rank correlations were determined and the data is presented in Table 19. Correlations of PC with the two AOA parameters were significant in different cooking methods and also they were comparable across the methods.

## 5 Calcium and vitamin D metabolism in periodontitis disease

The etiology of periodontal disease is believed to be multifactorial, but the exact factors involved have not been established. This disease is more common in aged persons. A paucity of information exists on the possible role of mineral and vitamin nutrition may play in periodontal disease. Marginal intakes of dietary calcium have been implicated as an etiological factor. Some of the risk factors can be modified to reduce a patient's susceptibility to periodontium. Calcium (Ca) is the major mineral present in osseous tissues. Deficiency can lead to a decrease in serum Ca resulting in mobilization from host tissues. Ca is needed for normal bone metabolism. A recent epidemiological study suggested that low dietary intake of Ca results in more severe periodontal disease. Low levels of dietary Ca were found to be associated with periodontal disease in all age groups. Further studies are needed to define the role of Ca and vitamin D in periodontal disease and to determine the extent to which Ca supplementation can modulate periodontal disease and tooth loss. Further, there are no Indian studies on the effects of supplementation of nutrients like Ca and Vitamin D on periodontal disease.

In fact, Vitamin D is synthesized in the skin during exposure to ultraviolet light and is also available in the diet, principally from oily fish. It is readily metabolized in the liver to form 25-hydroxyvitamin D (25OHD), the accepted measure of vitamin D status. Serum 25OHD concentrations tend to fall with age-related decline in the production of 7-dehydrocholesterol in the skin. The degree to which aging affects the absorption and metabolism of orally consumed vitamin D is not clear. But, supplements are required to improve serum vitamin D concentrations and the people requiring vitamin D supplementation consume it in the form of a daily tablet. In spite of the supplementation, the doses used are frequently inadequate, and that compliance with daily medication is likely to be sub-optimal. The long half-life of 25OHD was observed following oral calciferol supplementation means that larger less frequent doses are a practical alternative for the daily supplementation. There is no sufficient data available in the literature on the aspect of what and how the doses to be given in the above said clinical situation. Although single, large oral doses were studied but no data available on the frequency of optimum dosage. It is of interest to learn that, in the reported two studies a single high oral dose of vitamin D<sub>2</sub> produced similar vitamin D increases in young and old subjects and a smaller increase in 25-hydroxyvitamin D (25OHD) of old compared with young subjects in a third study. A report showed decrease in fractures with dosing of 1 00 000 IU cholecalciferol every 4 months. However, no studies have measured the time course of serum calcidiol concentrations after a large oral dose of cholecalciferol. Thus, it is the need of the hour to study the biochemical aspects of the single large dose in the degree of elevation of serum calcidiol during a specific duration. Hence, the present study was addressed the importance of the duration and the dosage of the Vitamin D in a normal human clinical situation of the old age group. During the process of the vitamin D dose

supplementation along with the levels of Vitamin D and Ca, an attempt was made to find the circulating status of associated nutrients like Mg, ZN and Cu.

## **AIMS AND OBJECTIVES**

It was hypothesized that the periodontal disease in Indian subjects might be associated with and/or due to the hypocalcaemia and vitamin D deficiency status.

## **METHODOLOGY**

### **A. Part-I**

The dose of Vit D was standardized in order to supplement Vitamin D in periodontitis disease subjects. About thirty normal subjects were recruited and given one dose of calciferol (60,000 I.U) with 500 mg Calcium per day for 60 days. Before the supplementation of calciferol, baseline blood samples were collected for the estimation of Vitamin D. After administering 60,000 IU of vitamin D, blood samples were drawn at an interval of 8 hrs, 24 hrs, 48 hrs, one week, two weeks, one month and two months. These samples were quantified for Vitamin D levels to determine the duration of vitamin D sufficiency after high dose, and to fix the frequency at which vitamin D could be supplemented along with calcium daily in the chosen subjects with Periodontitis.

### **B. Part-II**

The patients were recruited from Govt. Osmania Dental College, Hyderabad for the supplementation of Ca (500mg/day) and Vit.D (60,000 I.U/per month). The supplementation was continued for one year. Blood samples were drawn before supplementation for basal, 6 months and one year for the analysis of different biochemical parameters.

#### *Inclusion criteria*

1. Adult patients between >50 years, either sex.
2. Patients diagnosed as chronic periodontitis patients.
3. Patients having at least one molar with minimum pocket depth  $\geq 6$ mm in each quadrant of mouth.

#### *Exclusion criteria*

1. Patients with gross oral pathology.
2. Patients who were taking antibiotics.
3. Patients with systemic disease.
4. Patients who had received any periodontal treatment within six months prior to study.

### **C. Biochemical parameters:**

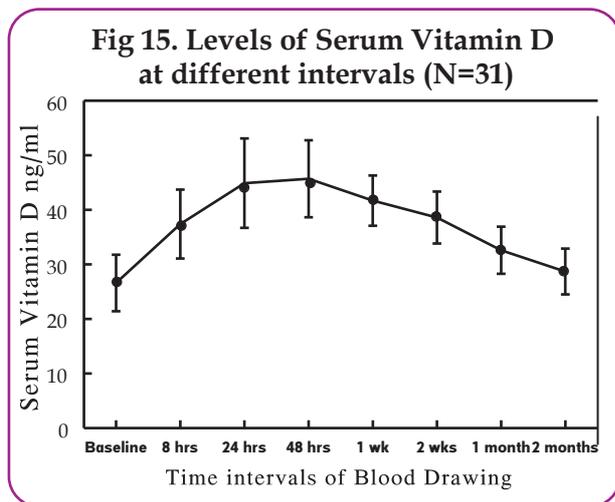
Blood samples were collected from the periodontal patients after supplementation once in six months for biochemical parameters analysis. They were analyzed for the following biochemical parameters using standard methods appropriately. Calcium, Magnesium, Zinc (by AAS), 25-OH-D3 (by radio immuno assay).

## **RESULTS**

### **A. Part I: Levels of Vitamin D, Ca and PTH (Baseline to 2 months):**

A single dose of Vitamin D of 60,000 IU was given to the participants and the blood samples were collected at different time intervals and studied for the three important biochemical parameters related to the vitamin D metabolism like Serum Vitamin D, Serum calcium and Serum Parathyroid Hormone levels. Fig 15 indicates the varied alterations in the vitamin D levels. At baseline the levels are found to be 26.6 ng/ml, these levels were increased after 8 hrs to 37.4 ng/ml; and to 44.9 after 24

hrs of the vitamin D dose. The increase in the levels of serum vitamin D levels were continued for 48 hrs and the levels are found to be a maximum of 45.7 ng/ml. The trend seems to decrease gradually after one week. The levels at 24hrs to 8 weeks 41.7, 38.6, 32.6 and 28.7 ng/ml respectively. The maximum saturation point at 48 hrs was observed to 45.7. The table 20 indicated the levels of Serum Calcium and PTH levels. These values found to be stable through out the study. The levels were 9.0 mg/dl for Serum calcium and 32.5 pg/ml at an average. There was no significant increase or decrease in the values for both of these parameters unlike the Vitamin D levels.



**Table 20. The levels of calcium and PTH at different intervals of time till two months (N=31)**

Time period	Serum Calcium mg/dl	Serum PTH pg/ml
Baseline	9.0 ± 0.3	32.1 ± 6.28
8 hrs	9.0 ± 0.2	32.1 ± 4.69
24 hrs	9.1 ± 0.27	33.6 ± 5.05
48 hrs	9.0 ± 0.26	32.5 ± 5.8
One week	9.0 ± 0.24	32.5 ± 6.04
Two weeks	9.0 ± 0.32	31.7 ± 5.55
One month	9.1 ± 0.29	31.4 ± 5.37
Two months	9.0 ± 0.32	31.2 ± 5.8

## B. Part II: Levels of Vitamin D, Ca, Zn, Mg and Cu (Baseline to One year) in Periodontitis subjects

The results indicated that the levels of Vitamin D were significantly increased at from 15.7 ng/ml to 33.3 ng/ml at the end of one year. At 6 months values are found to be 26.4 ng/ml. Similarly Ca levels were significantly increased by 6 months from 7.9 mg/dl (baseline) to 9.5 mg/ml. The values are found to be maintained till one year (9.3 mg/dl). Similarly, the circulating levels of Mg, Zn and Cu are found to be increased at 6 months (1.996 ng/dl, 99 µg/dl and 110 µg/dl respectively) when compared to the baseline levels (1.7 ng/dl, 97.35 µg/dl and 97.2 µg/dl respectively) but there was no substantial increase in levels were observed at one year interval (Table 21).

**Table 21. Levels of Vitamin D, Ca, Mg, Zn and Cu (Baseline to one year)**

Time	Baseline	6 months	One year
Vitamin D ng/ml	15.7 ± 0.4	26.4 ± 1.3	33.3 ± 1.3
Calcium mg/dl	7.9 ± 0.9	9.5 ± 0.43	9.5 ± 0.9
Magnesium ng/dl	1.7 ± 0.3	1.9 ± 0.7	1.9 ± 0.1
Zinc µg/dl	97.35 ± 2.3	99 ± 1.38	100 ± 2.1
Copper µg/dl	97.2 ± 1.9	110 ± 2.3	110 ± 3.3

### Clinical measurements of Periodontitis after supplementation of Vit.D and Calcium

The clinical measurements such as percentage of bleeding on Probing, Plaque Index, Gingival Index, Loss of attachment and Pocket depth were investigated (Table 22). The results indicated that upon supplementation of 60,000 IU/month of Vitamin D and 500mg/day of Calcium there was a decrease in the progress of the periodontitis disease indicators. The percentage of bleeding on probing was decreased from its basal value of 69.5 to 16.0 in a span of one year. Similarly, other important indicators such as plaque index and Gingival index were reduced from 1.8 to 1.01 and 2.1 to 0.73 respectively. The loss of attachment was also found be decreased from 3.53 to 2.27.

**Table 3. Clinical measurements of Periodontitis after supplementation of Vit. D and Ca**

S.No	Character	Basal (N=30)	6 months (N=25)	One year (N=25)
1	% of Bleeding on Probing	69.5 (53.1-100)	32.9 (15.6-70)	16.0 (0-50.4)
2	Plaque Index	1.8 (1-3)	1.7 (1-2)	1.01 (0-1.9)
3	Gingival Index	2.1 (1-3)	1.27 (0-2.3)	0.73 (0-1.7)
4	Loss of attachment (mm)	3.53 (1-6.3)	2.82 (1-4.1)	2.27 (1-3.5)
5	Pocket depth (mm)	4.37 (1-6.2)	3.91 (1-5.2)	3.23 (1-4.3)

The values expressed are in Averages (numbers in parenthesis shows the minimum and maximum values).

## CONCLUSIONS

In normal subjects, the supplementation of Vita D(60,000 IU) and Ca (500 mg/day) indicated a saturation point at 48 hrs and the circulating levels of Vit.D were gradually declined. The particular dose was selected and supplemented once in a month in the periodontitis subjects. At the end of the study, the clinical parameters indicated that the decrease in the progression of perodontitis disease.

6

## Feasibility of using umbilical cord serum (UCS) as a potential source for the growth and maintenance of pancreatic culture rat islets and assessment of their marker functions in comparison to fetal calf serum (FCS)- *in vivo* and *in vitro*

Animal sera are a potential source of microbiological contaminants, notably mycoplasma, bovine viruses and other pathogens. Hence, for cellular and organ transplantation use of calf / bovine serum supplement has not been recommended. Human UCS has been used as a serum replacement for FCS in propagation of several cell lines and has been found to be beneficial for growth and maintenance of primary cultures and cell lines, However, there are no studies to demonstrate the comparison of functional efficiency of the islets cells maintained in the primary cultures with and without HUCS *in vitro*. Further, assessing the viability and Insulin secretory function of the cryo-preserved islets is vital and has clinical application. A study was made to find the compatibility of HUCBS towards the maintenance of the islets isolated from WNINGR-Ob mutant rats (Mutants), their Lean (Lean) and parenteral controls (Controls) and their cytoprotection effects with Vit B6.

## AIMS AND OBJECTIVES

- ❖ Assessment of cell viability, integrity and their functional assessment in primary cultures of the islets maintained in the medium containing FCS, HUCS and HAB serum at different time points of the primary islet cell cultures (Mouse/Rat) and cell lines such as Panc-1 and HeLa.
- ❖ Cellular, molecular and functional characterization which includes cell cycle analysis, apoptosis and for the islets expression of specific transcriptional factors and its functional integrity by ELISA and Western Blot.
- ❖ Cryopreservation of the cultured islets and cell lines to check their viability at different time points of storage.

## METHODS

### *Islet isolation and culture*

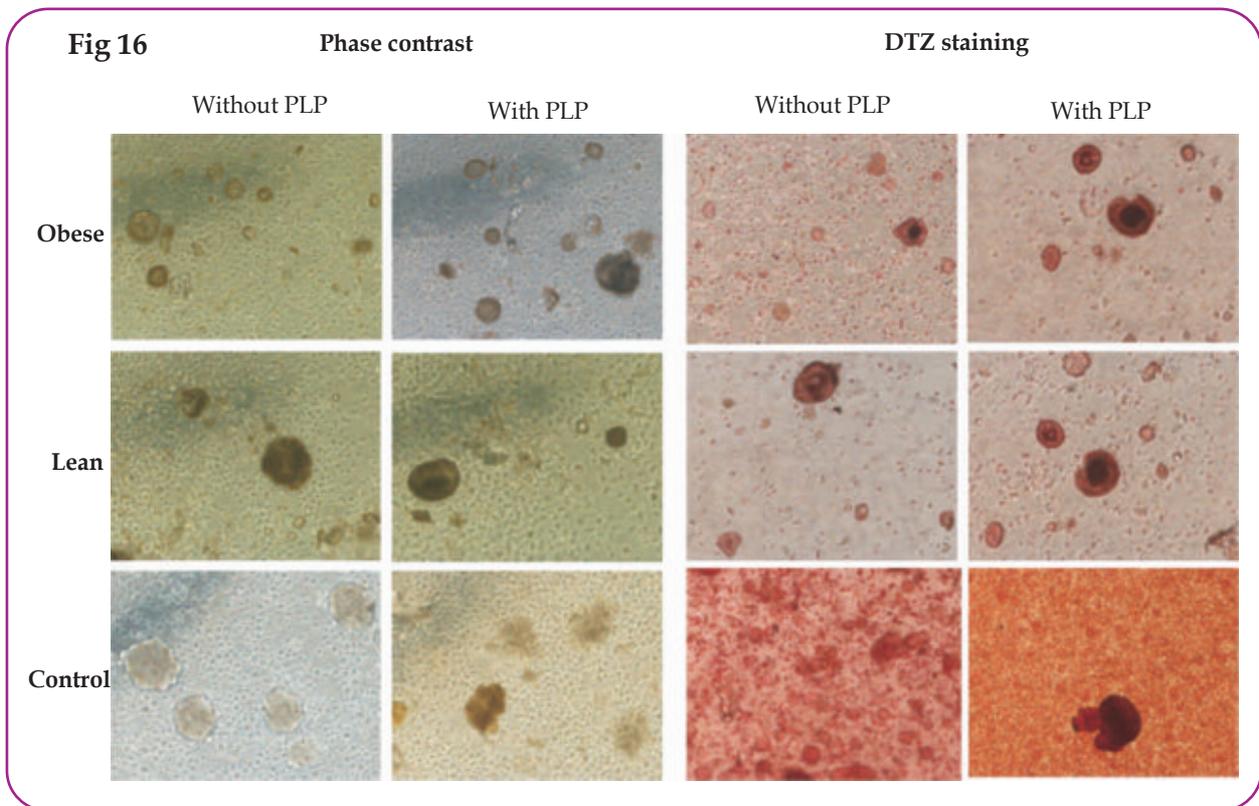
The pancreatic tissues were collected under aseptic conditions, minced in to very fine pieces, digested with filter sterilized collagenase solution as standardized in the Lab. The collagenase (0.1 mg-0.5 mg/ml) was used differentially for the Ob/Ob and for the WNIN animals. The isolated islets were seeded in to medium containing RPMI 1640 supplemented with 10% FBS (Gibco, grand Island, NY, USA) and Antibiotics (Penicillin 200 U/ml, and Streptomycin 0.2 mg/ml) in culture grade flask (corning USA) at 37° C and 5% CO2 for 48 hours. The islets were stained with DTZ and viewed under the microscope (Fig 16).

### *Cell Viability- (MTT) assay*

The primary cultures of the islets were assessed for their viability by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Briefly, MTT reagent (Sigma) was added to the islet cell suspension and incubated for a period of two hours at 37°C. The purplish blue colour after the addition of the formazan was measured at 532nm. The values have been expressed as percentage relative to the control cell samples.

### *Insulin secretion*

Islets from the Mutants, Lean and Controls were subjected to insulin secretion at basal (5.5 mmol/L) followed by high glucose challenge (16.5 mmol/L) in the presence and absence of PLP maintained in HUCBS. Insulin secretion at basal and with challenge was measured using a rat specific insulin ELISA kit (Biosource, Nivelles, Belgium). The values have been expressed as mIU/mg protein/h.



## RESULTS

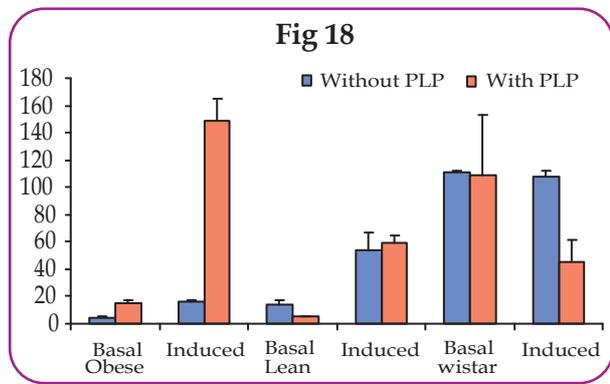
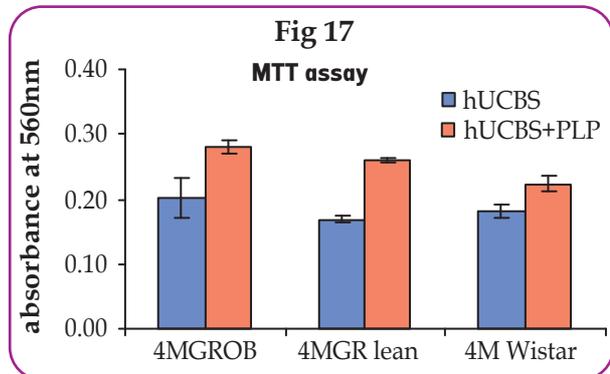
The isolated islets were seeded in to medium containing RPMI 1640 supplemented with 10% HUCS (Gibco, grand Island, NY, USA) and Antibiotics (Penicillin 200 U/ml, and Streptomycin 0.2 mg/ml) in culture grade flask (corning USA) at 37° C and 5% CO<sub>2</sub> for 48 hours. The islets were maintained for a period of 48 hrs stained with DTZ and viewed under the microscope.

### Cell Viability- (MTT) assay

The primary cultures of the islets maintained in HUCS with and without PLP showed viability >85% for Mutants, Lean and Controls (Fig 17).

### Insulin secretion

Islets from the Mutants, Lean and Controls were subjected to insulin secretion at basal (5.5 mmol/L) followed by high glucose challenge (16.5 mmol/L) in the presence and absence of PLP maintained in HUCBS. Insulin secretion was significantly increased with PLP priming and was evident on Mutants as compared to Lean (Fig 18).



## SALIENT FINDINGS

It was suggested that, hUCBS can be explored as an alternate serum supplement for FCS, making it more feasible in cell systems of human derived origin and can also find its application for the human transplantation programmes.

## 7. Pancreatic exocrine tissue as a source of progenitors/stem cells to generate insulin secreting cells. (Exploring the neurogenin positive cells as pancreatic progenitors from adult rodents (rat/mice))

The rat pancreatic tissue consists of 5-7% endocrine and 93-95% of exocrine fraction. The acinar cells were isolated from 3 months old male pancreatic tissue and cultured in Ham's F-12 with 10% FBS containing medium. The basic helix-loop-helix transcription factor neurogenin-3 (Ngn+3) is central player in the choice between exocrine and endocrine cell fate. It is a known fact that neurogenin-3 progenitor cell in the pancreatic acinar population most abundant and known to retain a remarkable plasticity both *in vivo* and *in vitro*. These acinar cells can undergo dedifferentiation *in vitro* where they loss their zymogene granules and gain duct like phenotype including marker cytokeratin-19. The molecular regulation of transdifferentiation from acinar to beta cells is known by growth factor like epidermal growth factor (EGF) presence of indomethacin and regulates Ngn+3, being the important transcriptional factor.

## OBJECTIVES

1. Isolation and characterization of acinar cells (Annual Report 2008-2009).
2. *In vitro* propagation and proliferation of pancreatic progenitors/stem cells (DEC, NPC and Ngn3+) from exocrine fraction of the cells.
3. Transdifferentiation of PP cells in to the insulin positive cells.

### *Work done during the year*

This study was aimed to isolate, characterize and explore the feasibility of Ngn3+ +ve cells from the acinar fraction, demonstrating amylase expression. It was subsequently assessed the viability by MTT assay, ROS generation and for protein expressions studies by BD FACS Aria II instrument. The acinar cells are highly fragile and explored the potential cytoprotective effects of vit B6 in the acinar primary cultures and have assessed the parameters either with or without Vit B6 (antioxidant).

## METHODOLOGY

### *A) Isolation and maintenance of primary cultures of Acinar cells*

Three months old male WNIN rats were selected for the experiments. The acinar cells isolation buffer briefly, 1mg/mL collagenase, 0.1% Trypsin EDTA, 104 mM NaCl, 5mM KCl, 2mM CaCl<sub>2</sub>, 1.2mM MgCl<sub>2</sub>, 25mM Hepes, 2.5mM D-Glucose, 0.1% BSA, 1mM KH<sub>2</sub>PO<sub>4</sub>. 0.01% non essential amino acid and 0.01% Soyabean Trypsin Inhibitor adjusted pH 7.4 has been used for digestion and washed and centrifuged to obtain the acinar cells enriched fraction. The acinar cells were seeded in the Hams F-12 with 10% FBS contained medium.

### *B) Viability assays*

The viability of cells was studied by MTT assay and values were recorded by ELISA multimode reader. The cells were treated with and without 5mM PLP incubated a period of 24 Hrs and cellular viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide intake was measured for viability of the cells. For the treatment of H<sub>2</sub>O<sub>2</sub> the 100 μM H<sub>2</sub>O<sub>2</sub> concentration was given for 30 minutes prior fixation of the cells.

### *C) Characterization*

The percentages of protein expressions were obtained from treated and untreated cells by FACS analysis briefly, fixed cells with 70% chilled ethanol and permeabilised with 0.05% triton 100X and 0.001% tween-20 solution. Blocking the cells with 2% BSA in PBS solution for 30 minutes. The percentages of basic protein amylase and transcription factors of nuerogenin-3 and PDX-1 expressions were obtained by FACS Diva software.

### *D) Antioxidant/cytoprotective effects of PLP*

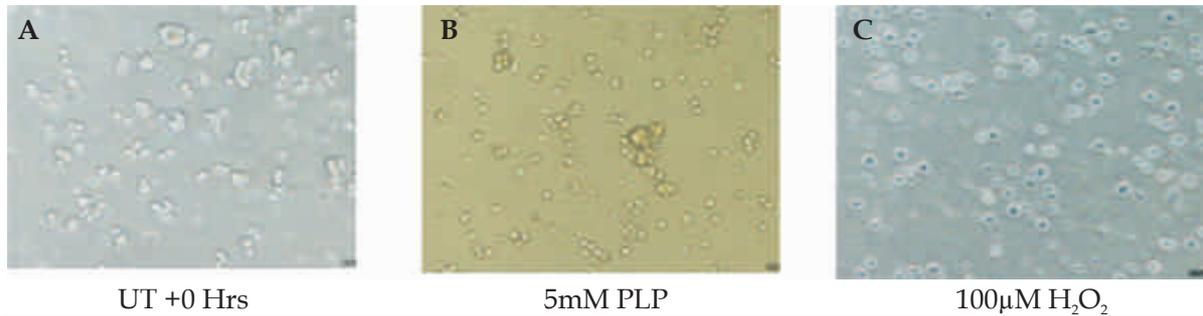
The antioxidant activity of acinar cells for control and treated cells were taken for cellular ROS, lipid peroxidation intensity has been expressed by relative fluorescence units (RFU) by ELISA method. Those cells were under stress were more for RFU value.

## RESULTS

### *Maintenance of primary cultures of Acinar cells with and without PLP*

Addition of 5mM PLP was protective to the acinar cellular integrity which was viewed Bright Field. However, cultures maintained with Hams F-12 with 10% FBS contained medium also gave similar results although treatment with H<sub>2</sub>O<sub>2</sub> resulted in cellular disintegration (Fig 19).

Fig 19

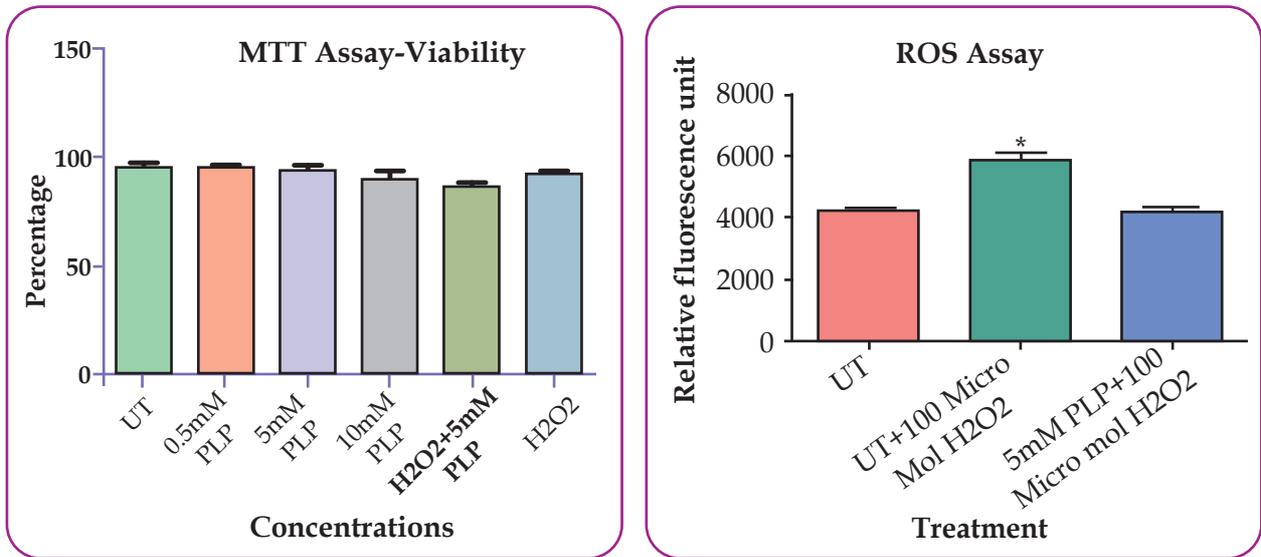


**Viability and ROS Assays**

There was no significant difference in the viability (MTT) at PLP treatments such as 0.5,5,10 mM of was given and 100µm H<sub>2</sub>O<sub>2</sub> treatments were given prior fixation of the cells. There was no significant difference was found. VMTT Assay for aciar cells Viability after 24 Hrs treatments. The different concentrations of 0.5,5,10 mM of PLP treatments were given and 100µm H<sub>2</sub>O<sub>2</sub> treatments were given prior fixation of the cells (Fig 20-23).

**Fig 20. (A) MTT Assay (viability):** The different concentrations of 0.5,5,10 mM of PLP treatments were given and 100µm H<sub>2</sub>O<sub>2</sub> treatments were given prior fixation of the cells. There was no significant difference was found.

(B) ROS assay performed with 5mM PLP was significantly normalized elevated levels of 100 µM H<sub>2</sub>O<sub>2</sub> generated stress measured in form of RFU values.



**Fig 21. The protein amylase expression in isolated acinar cells by FACS analysis showed increased % with 5, mM PLP, and sustained its level with H<sub>2</sub>O<sub>2</sub> treatment**

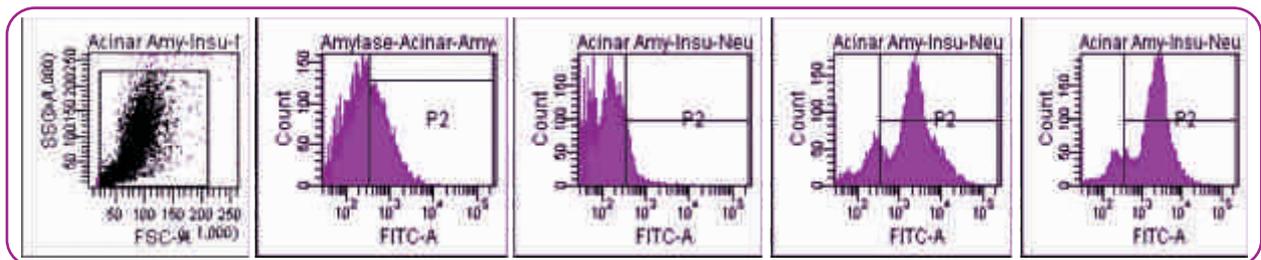


Fig 22. The specificity of PLP was further confirmed by the addition of its inhibitor AOAA which negated the effects of PLP (Table 1)

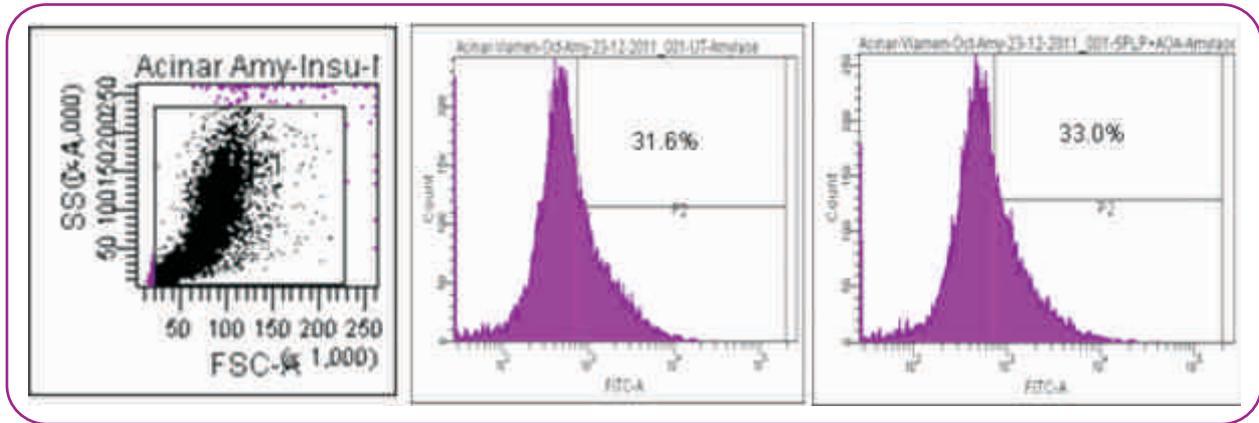
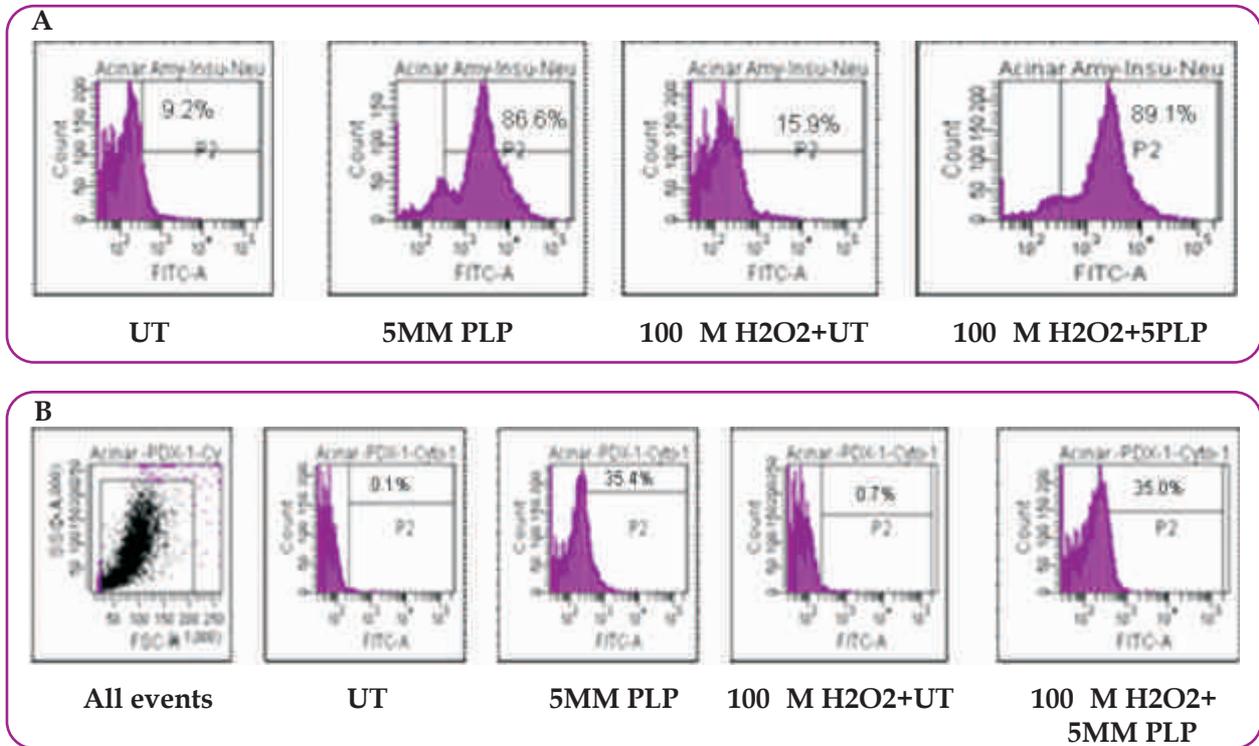


Fig 23. Shows that transcription factors Ngn3 (A) and PDX-1(B) were up regulated with H<sub>2</sub>O<sub>2</sub> treatment and Presence of PLP further increased



**SALIENT FINDINGS**

- ✎ Methodology has been standardized for acinar cultures.
- ✎ PLP addition was protective to acinar cells and demonstrated antioxidant effects with the addition of H<sub>2</sub>O<sub>2</sub>.
- ✎ PLP modulated the regulation of the transcriptional factors such as Ngn3, PDX-1, which are the master regulators for acinar lineage to beta cell formation.

This is the first study to show the beneficial effects of PLP on the exocrine fraction.

## 8 Exploration of basal glucocorticoid & levels and their possible role in obesity and insulin resistance using WNIN/Ob and WNIN/GR-Ob rat models

Glucocorticoids produced by adrenal cortex have divergent biological functions. Glucocorticoids play an important role in regulation of stress, intermediary metabolism, nervous and immune systems. Increased production of corticosteroids by the activation of Hypothalamus-Pituitary-Adrenal axis was observed in obesity and insulin resistance. Along with increased production, enhanced intracellular amplification of glucocorticoids by converting inactive glucocorticoids like cortisone [humans] and dehydrocorticosterone [rodents] into active cortisol and corticosterone respectively by action of enzyme 11beta-hydroxysteroid dehydrogenase type 1 was observed. The mechanisms by which corticosteroids induce obesity and insulin resistance are unclear.

### OBJECTIVE

To understand the possible role of glucocorticoids in development of obesity and insulin resistance in WNIN/Ob and WNIN/GR-Ob rat models.

#### *Work done during the year 2011-2012*

1. Identified the differentially-regulated genes in adipose tissue of WNIN/Ob obese rats using microarray.
2. Identified the differentially-regulated genes in liver of WNIN/Ob obese rats using microarray.

#### *Transcriptome analysis in adipose tissue of WNIN/Ob lean and obese rats*

Microarray analysis revealed that 1980 probe sets were differentially-regulated (more than two-fold) in adipose tissue of WNIN/Ob obese rats as compared with that of age and sex-matched lean rats. About 1017 probe sets were downregulated, whereas 963 probe sets were upregulated in adipose tissue of WNIN/Ob obese rats, as compared with those of lean rats.

Out of 1017 down regulated probe sets, 359 probe sets code for specific, known proteins. 365 probe sets (approximately 35% of the down regulated probe sets) were specific for non-coding RNA. Small nucleolar RNA makes major percentage of the non-coding RNA and also the highly downregulated genes in adipose tissue of WNIN/Ob obese rats. MicroRNAs (miRNA) were also present in the down regulated non-coding RNA genes. Other down regulated probe sets include those, which are specific for olfactory receptors, vomeronasal receptors and spetex proteins. Out of 963 upregulated probe sets, 787 probe sets codes for specific, known proteins. Remaining probe sets code for non-coding RNA and hypothetical proteins.

Majority of the upregulated genes (from 787 probe sets) were related to immune system and majority of the downregulated genes (From 359 probe sets) codes for structural proteins involved in cytoskeleton formation, cell to cell communication and extracellular matrix formation. Predicted cellular, metabolic and physiological changes based on the observed differentially-regulated genes in the liver of WNIN/Ob obese rats are given in Table 23. Some of the selected candidate genes that are well known to cause obesity and associated comorbidities are given in Table 24.

**Table 23. Predicted cellular, metabolic and physiological changes in retroperitoneal adipose tissue of four-month-old, male, WNIN/Ob obese rat based on the differentially regulated genes**

↑ Fatty acid biosynthesis	↑ Oxidative stress
↑ Triglyceride accumulation (endogenous synthesis and exogenous uptake)	↑ Cellular proliferation
	↑ Apoptosis
	↓ Preadipocyte differentiation
↓ Catecholamine induced-lipolysis	↑ Protein degradation
↑ Cholesterol accumulation (endogenous synthesis and exogenous uptake)	Altered cytoskeleton
	Altered cell-cell interactions
	Altered extracellular matrix composition
↑ Cholesterol uptake	Altered insulin like-growth factor (IGF-1) signalling
↑ Glycogen synthesis	↑ Infiltration of macrophages, NK cells and T cells
↑ Pathway	
↑ Leptin synthesis and secretion	
↑ Molecular stress (protein misfolding)	

**Table 24. List of selected candidate genes that are differentially-regulated in retroperitoneal adipose tissue of WNIN/Ob obese rat**

Category	Gene Symbol	Gene Name	Metabolic abnormality
Fatty acid/TG metabolism	<i>Scd1</i>	Stearoyl CoA desaturase 1 (?)	Obesity Insulin resistance
	<i>Elovl6</i>	Fatty acid elongase 6 (?)	Insulin resistance
	<i>Lipe</i>	Hormone -sensitive lipase (?)	Catecholamine induced-lipolysis
Receptors/Transcription factors	<i>Adrb3</i>	β3-Adrenergic receptor (?)	Catecholamine induced-lipolysis Obesity Insulin resistance
	<i>Esr1</i>	Estrogen receptor α (v)	Obesity
	<i>Ar</i>	Androgen receptor (?)	Obesity
	<i>Nr4a1</i>	Nuclear receptor subfamily group A, member 1 (?)	Obesity Insulin resistance
	<i>Adora1</i>	Adenosine A1 receptor (?)	Hyperleptinemia
	<i>P2rY1</i>	Purinergic receptor (?)	Hyperleptinemia
Cellular stress	<i>Gpx3</i>	Glutathione peroxidase 3 (?)	Systemic oxidative stress
Cell signaling	<i>Igf1</i>	Insulin like growth factor 1 (?)	Obesity
	<i>Igfbp3</i>	Insulin like growth factor-binding protein 3 (?)	Obesity
	<i>Igfbp5</i>	Insulin like growth Factor-binding protein 5 (?)	Obesity

↑increase ↓decrease

## 9 Effect of vitamin A on regulation of obesity in WNIN/Ob rat model

In simple terms, the obesity is defined as an abnormal metabolic condition resulting in excess storage of food-derived calories as triglycerides in white adipose tissue. This is caused by either excess calorie intake or decreased calorie expenditure or both. Genetic or environmental factors are known to play significant roles in the aetiology of this condition. Weight loss through diet and exercise is an effective way of containing the problem of obesity. However, this is easier said than achieved. Therefore, search was on for some novel pharmacological agents which can intervene at strategic points of development of obesity such as food intake, fat absorption, thermogenesis, the fat metabolism and its storage and the central controlling processes that regulate body weight. Previously, it was reported that vitamin A supplementation at the dose of 129mg/Kg diet for 2 months period, significantly reduced the body weight gain, adiposity index and retroperitoneal adipose tissue mass in 210days old male obese rats of WNIN/Ob strain and concluded that vitamin A could effectively bring down the adiposity after the complete development of obesity. This formed the basis for the current hypothesis that vitamin A supplementation at a very early age prevents the development of obesity.

### OBJECTIVE

To study the impact of chronic dietary vitamin A (high but non-toxic levels) supplementation (129mg/kg diet) on body weight gain and muscle fatty acid metabolism.

### METHODOLOGY

#### 1. Study design

50 days old male 16 lean and 16 obese rats of WNIN/Ob strain were taken and divided into two groups consisting of 8 lean and 8 obese rats in each receiving either 2.6mg or 129mg of vitamin A/kg diet for a period of 4 months (Table 25). Weekly body weight and food intake was recorded. At the end, blood was drawn, animals were sacrificed and various tissues were collected, weighed and stored in -80°C for further analysis.

Table 25

Experimental groups			
Lean		Obese	
AI	AII	BI	BII
Stock diet containing 2.6mg vitamin A/ kg diet	Vitamin A-enriched diet (129mg vitamin A/kg diet)	Stock diet containing 2.6mg vitamin A/ kg diet	Vitamin A-enriched diet (129mg vitamin A/kg diet)

#### 2. Soleus muscle lipids, fatty acid composition and protein expression

Using the lab standard protocol, soleus muscle total lipids were extracted and assayed for cholesterol and triglyceride (TG) levels. Phospholipid (PL) and TG fractions of total lipids were separated, analyzed for fatty acid composition by gas-liquid chromatography and fatty acid desaturation index was calculated.

For protein expression analysis, soleus muscles were homogenized in a Tris buffer containing 250 mM sucrose, 10 mM Tris (pH 7.4), 1 mM EDTA, 1 mM DTT supplemented with protease and phosphatase inhibitors cocktails. From the obtained cytosolic and microsomal fractions, 60g was used to detect the protein expression by ECL chemiluminescence method, using antibody against stearoyl CoA desaturase 1 (SCD1) in microsomal fraction. -actin was used to normalize the protein expression.

### 3. Statistical analysis

Results were expressed as mean±SE. Statistical significance was determined by one-way ANOVA and  $P \leq 0.05$  was considered significant. Data were analyzed using SPSS software package (Version 11.0).

### RESULTS

#### Effect of vitamin A on muscle lipids, SCD1 expression, fatty acid desaturation index and composition

Muscle triglycerides (TG) and cholesterol levels were higher in stock diet-fed obese rats as compared with lean counterparts. However, feeding of vitamin A-enriched diet had no impact on these parameters in both the phenotypes (Table 26).

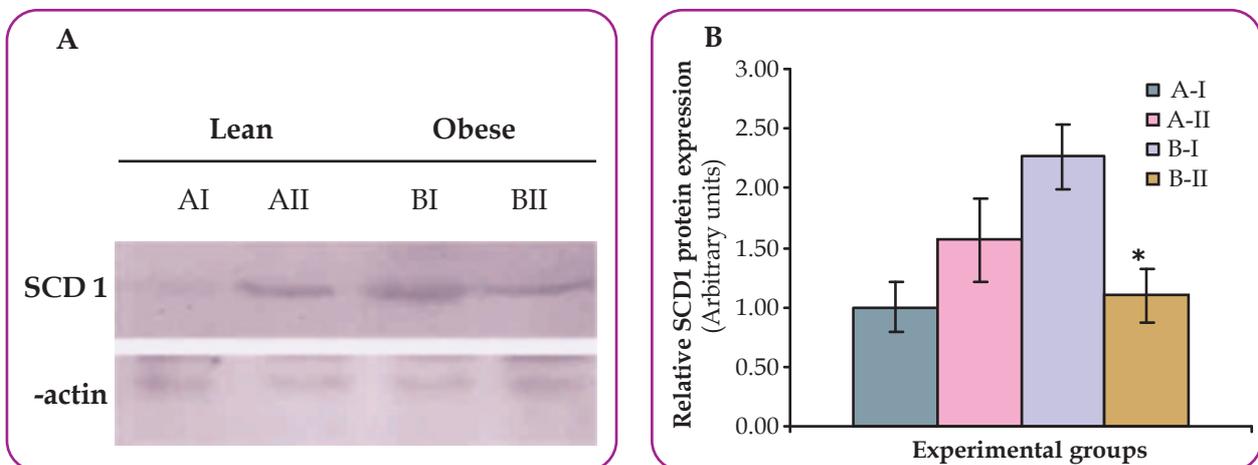
**Table 26. Effect of vitamin A on muscle lipids**

Soleus muscle lipid parameters (mg/g tissue)	Lean		Obese	
	A-I	A-II	B-I	B-II
Triglycerides	1.11±0.54	1.58±0.29	4.0±0.59	5.6±1.72
Cholesterol	0.45±0.05	0.62±0.21	0.9±0.1	1.2±1.1

Values are given as means ± SE of 4 rats. Vitamin A-enriched diet was compared to stock diet of respective phenotypes.  $p < 0.05$  was considered significant (one-way ANOVA). A-I, A-II-Stock diet & B-I, B-II-Vitamin A-enriched diet fed groups.

Western blot data showed no significant change in the expression of SCD1 protein in lean rats, however significantly down-regulated its expression in obese rats fed with vitamin A-enriched diet (Fig 24).

**Fig 24. Effect of vitamin A on SCD1 protein expression**



a. Representative Western blot showing the levels of soleus muscle SCD1 protein b. Histogram represents the densitometric (arbitrary units) values of blot relative to stock diet-fed lean rats. Values are given as means ± SE of 3-4 rats. Vitamin A-enriched diet was compared to stock diet of respective phenotypes.  $p < 0.05$  was considered significant (one-way ANOVA). A-I, A-II-Stock diet & B-I, B-II-Vitamin A-enriched diet fed groups.

Compared with stock diet feeding, fatty acid desaturation index, the ratio of palmitoleic to palmitic (16 : 1/16 : 0) of TG and phospholipid (PL) fractions showed significant decrease, especially with undetectable 16 : 1 levels of PL fraction of obese rats fed on vitamin A-enriched diet, with remarkable reduction in total monounsaturated fatty acids (MUFA) content. Furthermore, the ratio of oleic to stearic acid (18 : 1/18 : 0) remained unaltered in both these

fractions of vitamin A-enriched diet-fed lean and obese rat soleus muscle as compared with that of stock diet-fed respective rats (Fig 25).

**Fig 25. Effect of vitamin A on muscle fatty acid composition**

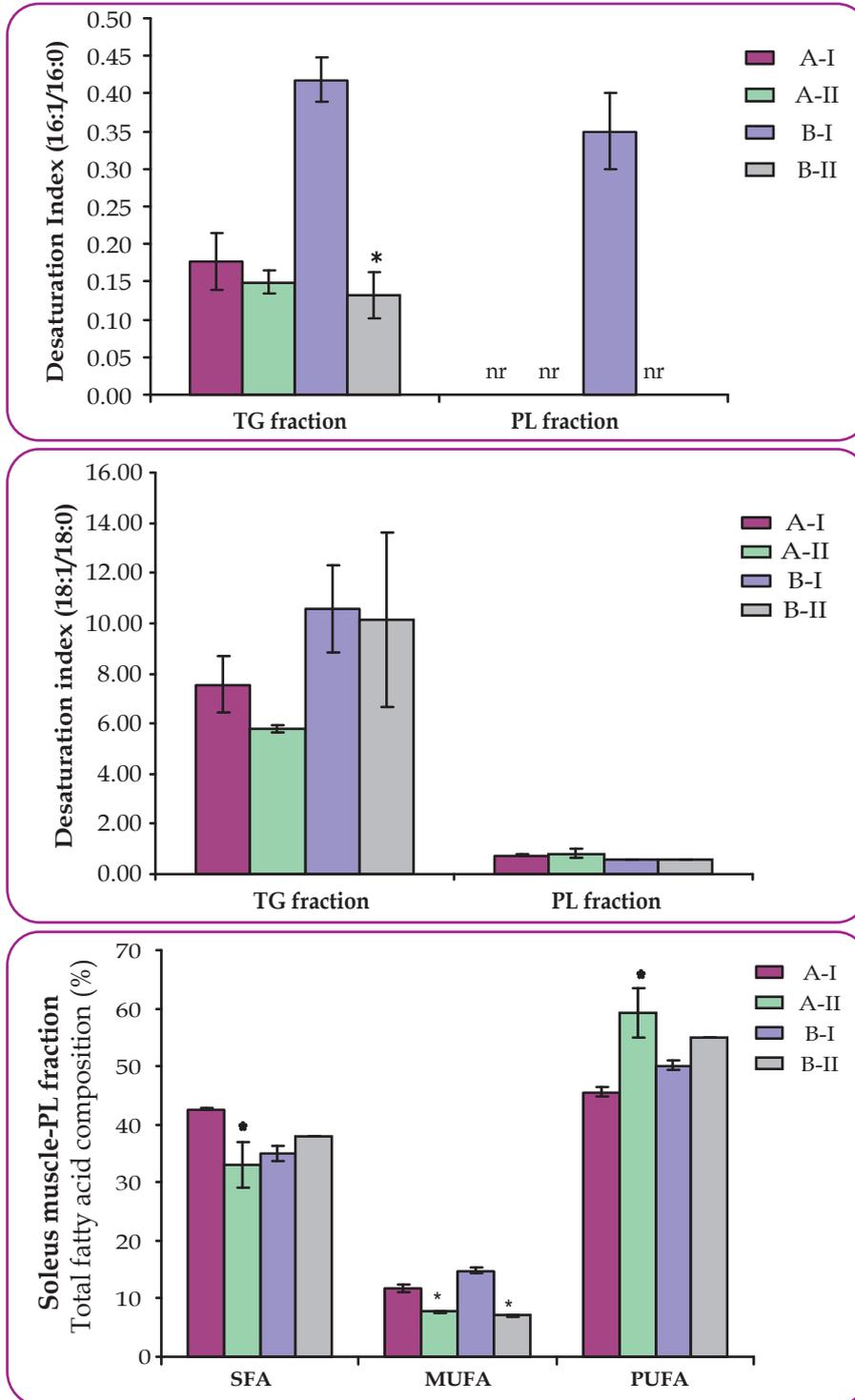


Fig. 25. Soleus muscle total lipid-TG & PL fraction fatty acid desaturation indices (Ratio of 16:1 to 16:0 & 18:1 to 18:0). nr-No ratio, as 16:1 level was undetectable. iii) Soleus muscle total lipid-PL fraction total fatty acid composition Values are given as means  $\pm$  SE of 3-4 rats. Vitamin A-enriched diet was compared to stock diet of respective phenotypes. p0.05 was considered significant (one-way ANOVA). A-I, A-II-Stock diet & B-I, B-II-Vitamin A-enriched diet fed groups.

**CONCLUSIONS**

Chronic vitamin A-enriched diet feeding to obese rats from an early age, not only ameliorates the development of obesity, but also improves the insulin sensitivity possibly by down-regulating protein tyrosine phosphatase 1B (PTP1B) levels of muscle. Finally, greater physiological benefits are accrued by early pharmacological/dietary intervention and also PTP1B seems to be a potential target for pathological conditions like obesity and insulin resistance.

## 10 Diabetic retinopathy-role of micronutrients: (ii) biochemical and molecular studies on the effect of vitamin-B<sub>12</sub> on retina under hyperglycemic conditions

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes and is a major public health problem considering the global prevalence of diabetes. Studies have shown that the prevalence of DR increases with diabetes duration and intensive glycemic control could delay the development of DR. However, multiple factors are likely to be involved in predisposing diabetic subjects to complications, as evidenced by the fact that many but not all diabetic patients develop diabetic complications. While a number of studies focused on the genetic susceptibility, role of other environmental factors such as nutritional and dietary factors in the development of DR has not been investigated. Supplementation of vitamins and minerals for the management of type-2 diabetes (T2D) though has been reported, the role of micronutrients in the prevention and development of T2D in general and diabetic complications in particular has not been established clearly.

Therefore, hospital based case-control study was conducted to understand the association of micronutrient status in DR patients. Based on the data obtained, on the levels of vitamins and minerals in diabetics with and without retinopathy in comparison with normal control subjects in these studies, it was found significantly that lower levels of vitamin B<sub>12</sub> in DR patients when compared with diabetics with no retinopathy. Further, a positive correlation was observed between low vitamin B<sub>12</sub> and increased homocysteine levels in DR patients. Therefore, lower vitamin-B<sub>12</sub> status appears to be a determining factor for increased homocysteine in DR in this study. Further, vitamin-B<sub>12</sub> levels seem to be not influenced by age, BMI and duration of diabetes. Together, these results suggest that vitamin-B<sub>12</sub> deficiency could be an independent risk factor for DR.

However, there are no studies that explain the possible role of vitamin B<sub>12</sub> in the development of DR in experimental conditions. Hence, studies were carried out in animal models to understand the role of vitamin B<sub>12</sub> on the retina under diabetic conditions by biochemical and molecular investigations.

**METHODOLOGY**

Two-month-old male SD rats with an average body weight of  $250 \pm 12$  g were made into five groups: Group I (control), Group II (B<sub>12</sub> deficiency), Group III (diabetes), Group IV (diabetes with B<sub>12</sub> deficiency) and Group V (Diabetes with B<sub>12</sub> supplementation). Diabetes was induced with single intraperitoneal injection of streptozotocin (37 mg/kg) in citrate buffer. Animals in group I and group III received AIN-76A control diet containing B<sub>12</sub> content 0.016 g/kg diet, whereas group II

and group IV animals received AIN-76A diet containing B<sub>12</sub> content of 0.006 g/kg diet added with citrus pectin 50 g/kg diet (B<sub>12</sub> deficient diet) and group V animals received AIN-76A diet containing B<sub>12</sub> content 0.032 g/kg diet (B<sub>12</sub> supplement diet, 2X times of B<sub>12</sub> to normal diet). Animal care protocols were in accordance with and approved by the Institutional Animal Ethics Committee.

The animals were maintained on respective diets for four months after induction of diabetes. At the end of the experiment animals were sacrificed by CO<sub>2</sub> asphyxiation. From each group 4 eye balls from 4 rats were kept for formalin fixation to perform histopathology and from 4 rats, retina were dissected out from eyeballs, snap-frozen in liquid nitrogen and stored at -80°C for gene expression and protein status studies. Blood was drawn into anticoagulant tubes and red blood cells (RBC) were separated from plasma by centrifugation for the following analyses.

**Biochemical parameters:** Glucose, HbA1C, insulin and lipid profiles such as total cholesterol, triglycerides, HDL, LDL were estimated by kit methods.

**Determination of vitamins:** The levels of B<sub>12</sub> and folic acid and homocysteine in plasma were estimated simultaneously by dual count radio immunoassay and HPLC, respectively as described previously.

**Estimation of sorbitol and aldose reductase (ALR2) activity in RBC:** ALR2 activity and sorbitol in erythrocytes were estimated as described previously.

**Histopathology:** For histological studies, eye balls were dissected out and fixed in 10% neutral buffered formalin overnight. The tissues were later paraffin embedded and blocks were prepared. Hematoxylin and eosin staining was done on 4µm paraffin sections. Retinal morphology was examined under the microscope.

**Expression of retinal genes by Real-Time PCR:** Total RNA was isolated from retinal tissue using TRI reagent. Total RNA was reverse transcribed by using high capacity cDNA reverse transcription kit. Quantitative real-time PCR was then performed on cDNA templates using gene specific primers. Relative change in mRNA expression was calculated by use of the  $2^{-\Delta\Delta CT}$  values. Genes implicated in retinal structural and functional integrity such as Vegf, Ngf, Gfap, Hif-1 $\alpha$  and rhodopsin expression at mRNA level was measured in different groups of rats as described previously.

**Western blot analysis:** Retina was homogenized in lysis buffer containing 50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate and 0.1% triton-X-100. The homogenate was centrifuged at 8000 rpm for 10 minutes and supernatant was used as total retinal lysate. Retinal lysate was resolved under reducing conditions on 12% SDS-PAGE, proteins were transferred onto a nitrocellulose membrane (NC) and incubated with respective primary antibody later with HRP-conjugated goat anti-rabbit antibody. Subsequently, detection was performed with diaminobenzidine in the presence of hydrogen peroxide.

## RESULTS

- 1. Fasting blood glucose and insulin:** Blood glucose levels were elevated and insulin levels were lowered significantly in diabetic groups compared with control group (Table 27). These results were expected due to the destruction of pancreatic  $\beta$ -cells by streptozotocin, a well-known mechanism used for induction of diabetes in rodents. Nonetheless, supplementation with B<sub>12</sub> diet did not normalize blood glucose and insulin levels (Table 27). Interestingly, an elevated level of fasting glucose was observed in rats fed with B<sub>12</sub> deficient diet (group II). Thus, this study reports for the first time that vitamin B<sub>12</sub> deficient diet affecting the blood glucose levels in SD rats. However, the mechanism how vitamin B<sub>12</sub> deficient diet induced alteration in blood glucose levels is not known.

**Table 27. Glucose and insulin levels at two and four months of the experiment**

Groups	Two months		Four months	
	Glucose (mg/dL)	Insulin ( $\mu$ U/mL)	Glucose (mg/dL)	Insulin ( $\mu$ U/mL)
Group I	80.7 <sup>a</sup> ±4.1	36.1 <sup>a</sup> ±3.1	83.8 <sup>a</sup> ±7.2	36.1 <sup>a</sup> ±3.1
Group II	219.0 <sup>b</sup> ±55.7	17.2 <sup>b</sup> ±0.7	149.8 <sup>b</sup> ±48.2	13.2 <sup>b</sup> ±1.4
Group III	349.5 <sup>b</sup> ±59.7	9.7 <sup>b</sup> ±4.5	293.7 <sup>b</sup> ±59.1	7.6 <sup>c</sup> ±3.8
Group IV	304.6 <sup>b</sup> ±68.1	14.3 <sup>b</sup> ±1.5	298.0 <sup>b</sup> ±61.7	12.5 <sup>b</sup> ±1.5
Group V	296.0 <sup>b</sup> ±118	13.8 <sup>b</sup> ±0.8	231.0 <sup>b</sup> ±87.1	12.3 <sup>b</sup> ±2.7

Values are mean  $\pm$  SD (n=8), where group I (normal), group II (B<sub>12</sub> deficiency), group III (diabetic), group IV (diabetic with B<sub>12</sub> deficiency), and group V (diabetic with B<sub>12</sub> supplementation). Means with different superscripts are significantly different (P<0.05).

- Lipid profiles:** There were no significant alterations in lipid profiles such as total cholesterol, triglycerides, LDL and HDL cholesterol between groups.
- Plasma vitamin B<sub>12</sub>, folic acid and homocysteine levels:** B<sub>12</sub> and folic acid levels were measured in plasma at second (midpoint) and fourth month (end) of the experiment. Two months feeding of varying B<sub>12</sub> diets showed the expected trend in B<sub>12</sub> status based on plasma levels (Table 28). Further, continued feeding of normal, B<sub>12</sub> deficient, and B<sub>12</sub> supplement diets in corresponding groups for four months followed the same trend. Supplementation (group V) showed normal levels of plasma B<sub>12</sub> and B<sub>12</sub> deficient group of rats showed 40-60% decrease in B<sub>12</sub> levels compared with control group (Table 28). In all the groups however, folic acid levels were not altered at two and four months (Table 28). There was a significant increase in plasma homocysteine levels in B<sub>12</sub> deficient group (group II) compared with group I. Significant increase in homocysteine was also observed in group III and group IV. B<sub>12</sub> supplementation (group V) showed a homocysteine levels comparable with control group (Table 28).

**Table 28. Levels of vitamin B<sub>12</sub>, folic acid and homocysteine**

Groups	Two months		Four months		
	Vitamin B <sub>12</sub> (pg/mL)	Folic acid (ng/mL)	Vitamin B <sub>12</sub> (pg/mL)	Folic acid (ng/mL)	Homocysteine ( $\mu$ mol/L)
Group I	485.0 <sup>a</sup> ±73.1	19.8 <sup>a</sup> ±3.1	421.6 <sup>a</sup> ±41.6	16.0 <sup>a</sup> ±2.2	5.75 <sup>a</sup> ±1.12
Group II	127.5 <sup>bc</sup> ±42.5	24.1 <sup>a</sup> ±2.6	60.0 <sup>b</sup> ±15.0	18.9 <sup>a</sup> ±0.7	16.3 <sup>b</sup> ±3.58
Group III	438.0 <sup>ab</sup> ±124.7	20.4 <sup>a</sup> ±2.5	228.0 <sup>c</sup> ±55.4	13.8 <sup>b</sup> ±3.8	9.9 <sup>c</sup> ±1.26
Group IV	121.0 <sup>c</sup> ±35.0	23.8 <sup>a</sup> ±3.0	84.0 <sup>bc</sup> ±15.0	14.1 <sup>ab</sup> ±1.9	13.65 <sup>bc</sup> ±2.87
Group V	538.3 <sup>a</sup> ±53.0	22.0 <sup>a</sup> ±2.5	558.3 <sup>a</sup> ±171.5	21.5 <sup>c</sup> ±1.0	6.47 <sup>ac</sup> ±1.02

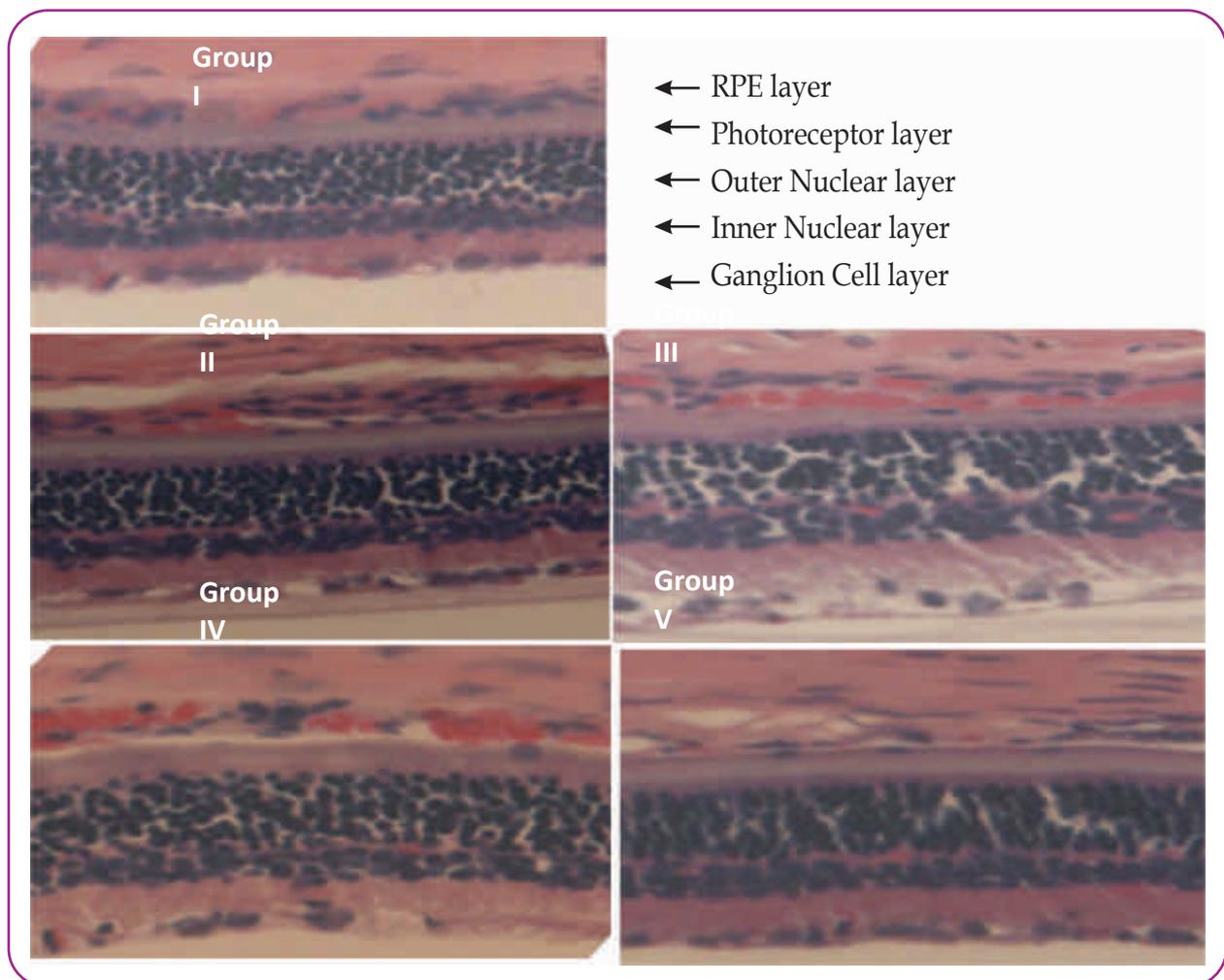
Values are mean  $\pm$  SD (n=8). Group I (normal), group II (B<sub>12</sub> deficiency), group III (diabetic), group IV (diabetic with B<sub>12</sub> deficiency), group V (diabetic with B<sub>12</sub> supplementation). Means with different superscripts are significantly different (P<0.05).

- Aldose reductase activity and sorbitol levels:** Activity of ALR2, a key enzyme of the polyol pathway, was significantly higher in all diabetic groups (III-V) compared with group I. Further, sorbitol levels were also showed similar trend thus supporting the activation of polyol pathway under hyperglycemic conditions. However, supplementation of B<sub>12</sub> diet to diabetic rats did not normalize polyol pathway. Further, there was no significant difference in the ALR2

activity and sorbitol levels between group I and II suggesting the B<sub>12</sub> deficiency *per se* may not affect polyol pathway.

- Morphological evaluation of retina:** Morphological observations indicate micro vascular leakage and accumulation of lipofuscin droplets appearance on the epithelial layers of diabetic and B<sub>12</sub> deficient groups (Fig 26). Notable finding was that presence of vascular leakage and lipofuscin accumulations in B<sub>12</sub> deficient rats (Fig 26). Observation of different retinal layers across the groups indicate abnormalities in cell number of GCL (ganglion cell layer) and structural distortions in outer nuclear layer of diabetic and B<sub>12</sub> deficient groups compared to control group (Fig 26). Interestingly, retina of diabetic rats supplemented with B<sub>12</sub> diet showed morphology comparable to control with no accumulations of lipofuscin molecules and absence of any leaking areas, well integrated layers and cells.

**Fig 26. Retinal morphology.** Hematoxyline and Eosine stained retinal sections of Group I (normal), group II (B<sub>12</sub> deficiency), group III (diabetic), group IV (diabetic with B<sub>12</sub> deficiency), group V (diabetic with B<sub>12</sub> supplementation)



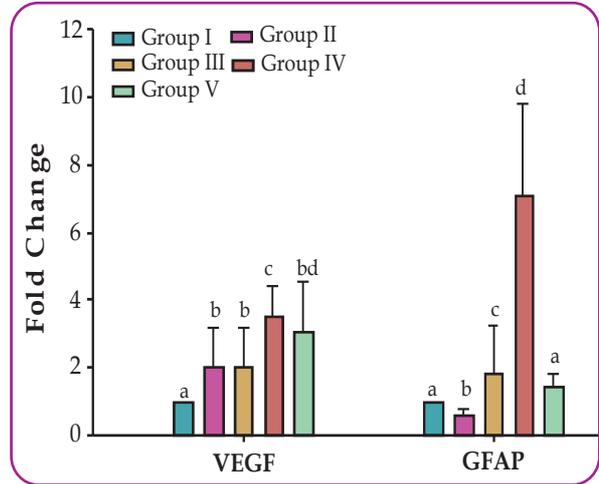
- Gene expression studies:** To study the effect of B<sub>12</sub> deficiency and supplementation on retina under normal and hyperglycemic conditions, expression of markers of retinal pathogenesis such as vascular endothelial growth factor (VEGF), nerve growth factor (NGF), glial fibrillar acidic protein (GFAP), hypoxia inducible factor (HIF-1 $\alpha$ ) and *rhodopsin* was analyzed.

A significant increase of VEGF expression in B<sub>12</sub> deficient (group II) and diabetic groups (group III, IV and V) compared with control group (group I) was seen. B<sub>12</sub> deficient rats and diabetic rats showed 2 fold increase in expression, whereas diabetic rats fed with B<sub>12</sub> deficient diets (group IV) showed 3.5 fold increase in expression compared with control group (Fig. 27). However, B<sub>12</sub> supplementation to diabetic group (group V) showed 3 fold increases in expression compared with control group (Fig. 27) indicating unaltered effect of B<sub>12</sub> supplementation on VEGF expression. Expression of stress response protein GFAP was increased significantly to 1.8 folds in group III and 7.19 fold increase was observed in group IV compared to group I, whereas no significant difference observed in GFAP between group II compared with group I (Fig 27). Interestingly, B<sub>12</sub> supplemented diabetic rats (group V) showed normal expression as compared with control group (Fig 27).

There was a decrease in rhodopsin expression in group II, III and further decrease was observed in group IV compared with group I (Fig 28). B<sub>12</sub> supplementation to diabetic rats (group V) showed a normalized expression of rhodopsin compared with control indicating the normal structural integrity of photoreceptor layers. Expression of HIF-1 $\alpha$  showed no significant difference between control and other four groups (group II-V) (Fig 28). The indicator of pericyte cell marker, NGF expression decreased 2 fold in group II, III and IV compared with group I. B<sub>12</sub> supplementation to diabetic rats (group V) showed a marginal decrease in the expression of NGF compared with control (group I) indicating the protective effect of B<sub>12</sub> supplementation by preventing the loss of pericytes in these group of rats (Fig 29).

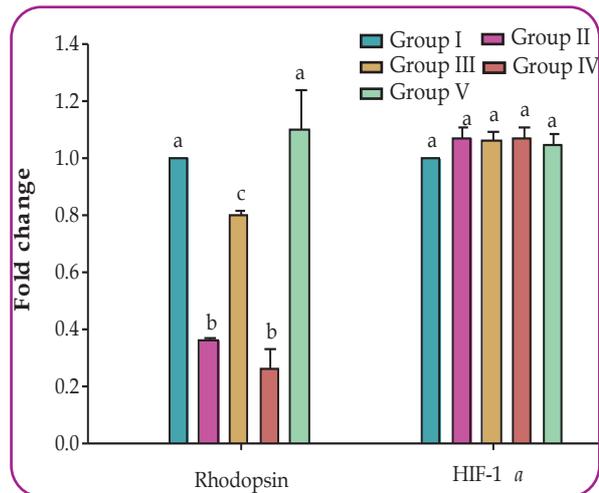
**7. Protein expression studies:** Immunodetection of retinal lysates was performed with selected markers to validate the

**Fig 27 Gene expression pattern of VEGF and GFAP.** Transcript levels of VEGF and GFAP in retina by real-time PCR. Group I (normal), group II (B<sub>12</sub> deficiency), group III (diabetic), group IV (diabetic with B<sub>12</sub> deficiency), group V (diabetic with B<sub>12</sub> supplementation).



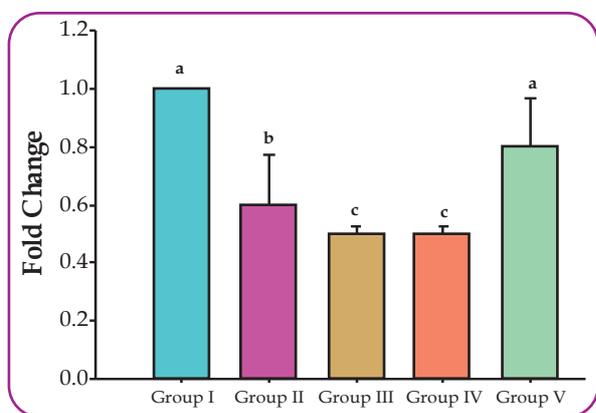
The data are mean  $\pm$  SD (n=4). The bars that do not share common superscripts are significantly different from each other at (P<0.05).

**Fig 28. Gene expression pattern of rhodopsin and HIF-1 $\alpha$ .** Transcript levels of rhodopsin and HIF-1 $\alpha$  in retina by real-time PCR. Group I (normal), group II (B<sub>12</sub> deficiency), group III (diabetic), group IV (diabetic with B<sub>12</sub> deficiency), group V (diabetic with B<sub>12</sub> supplementation).



The data are mean  $\pm$  SD (n=4). The bars that do not share common superscripts are significantly different from each other at (P<0.05).

**Fig 29. Gene expression pattern of NGF.** Quantification of NGF in retina by real-time PCR. Group I (normal), group II ( $B_{12}$  deficiency), group III (diabetic), group IV (diabetic with  $B_{12}$  deficiency), group V (diabetic with  $B_{12}$  supplement).



The data are mean  $\pm$  SD (n=4). The bars that do not share common superscripts are significantly different from each other at ( $P < 0.05$ ).

expression of VEGF, NGF, GFAP and rhodopsin genes at protein level. Altogether, immunodetection data validate gene expression observations of real-time PCR.

### CONCLUSIONS

These results indicate a role for vitamin- $B_{12}$  in retinal structure and function both in neuronal and vascular component particularly under hyperglycemic conditions. Thereby, these data provide a molecular basis for vitamin- $B_{12}$  deficiency associated with diabetic retinopathy.

Furthermore, an interesting finding of this study was that high glucose levels in  $B_{12}$  deficient rats and supplementation of vitamin  $B_{12}$  has resulted in significant beneficial outcomes in normalizing neuronal, vascular and inflammatory mediators under hyperglycemic conditions in the retina at the molecular levels.

## 11 Impact of agents with potential use in functional foods on biomarkers for induction of age related diseases: Inhibition of protein glycation by ellagic acid

Cancer and cardiovascular diseases associated with atherosclerosis and diabetes constitute the major causes of age related diseases. A number of epidemiological studies have consistently demonstrated the protective effects of fruits and vegetables with respect to age related diseases such as diabetes, atherosclerosis and several types of cancer. In Europe, this association seems especially obvious with respect to the protective action of the traditional Mediterranean-style diet, and where antioxidant constituents in red wine also appear to play a significant role. The crucial issue that this project attempts to tackle the following aspects. While beneficial effects have been firmly established, it has not been possible to identify exactly which components in food are mainly responsible for the protective action with respect to age-related diseases, mainly because of the complex exposures involved, as well as the interaction with genetic and other confounding factors. The main aim of this project was to investigate the protective action of agents with potential use as functional food constituents with respect to cancer, diabetes and cardiovascular disease. In collaboration between EU and Indian Research Centers, the proposal features a multipronged approach, where the protective action of various non-toxic agents were studied *in vitro* as well as in rodent models with respect to induction of biomarkers for the development of diabetes and diabetic complications.

The long-term complications of diabetes are thought to be a result of the accumulation of tissue macromolecules that have been progressively modified by non-enzymatic glycation. Glycation is a complex series of covalent chemical reactions between the carbonyl group of

reducing sugars and the amino group of proteins. *In vivo*, a diverse array of advanced glycation endproducts (AGE) have been detected and some of which are characterized as N-epsilon carboxymethyllysine (CML), N-epsilon carboxyethyllysine (CEL), pentosidine, argpyrimidine, imidazolysines, etc. Glycation alters structure and function of many proteins as it changes protein charge network, secondary and tertiary structure. Formation of AGE plays a key role in the development of several pathophysiologicals associated with aging and diabetes such as atherosclerosis, chronic renal insufficiency, Alzheimer's disease, nephropathy, neuropathy and cataract. This raises the possibility that inhibition of AGE formation may prevent the progression of diabetic complications. However, designing a drug having anti-AGE activity was a challenge due to the complexity of reactions involved in the formation of AGE.

Ellagic acid (EA; 2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione) is found in numerous fruits and vegetables and other plant foods. EA is known to have antioxidant, anti-inflammatory and anticarcinogenic properties. Earlier studies showed that, aqueous extracts of some traditional medicines and common dietary agents such as fruits, vegetables, spices and herbs have inhibited or prevented AGE formation under *in vitro* conditions. Apart from the greatest sources like berries and pomegranate, EA is also present in those dietary sources to have antiglycating potential such as apples, grapes, orange, guava and cumin. Therefore, in the present study, the antiglycating activity of EA and its mechanism of action using different *in vitro* protein glycation systems was investigated. Further, the significance and utility of EA antiglycating potential was strengthened in *ex vivo* physiological conditions.

## EXPERIMENTAL

***In vitro* glycation of proteins and inhibition with EA:** Each 1 mL reaction mixture contained 10 mg protein (total soluble protein, TSP from eye lens, hemoglobin (Hb), BSA or lysozyme) in 0.2 M phosphate buffer, (pH 7.4), 0.2 M fructose, 50 µg of penicillin and streptomycin and 0.01% sodium azide. Similarly, human hemoglobin (Hb; 20 mg) was glycated with 0.2 M fructose. For inhibition studies, various concentrations of EA (typically 50, 100, 150 and 200 µM) were added to *in vitro* protein glycation assay mixture and incubated in dark at 37° C for 1-3 weeks as described above. At the end of the incubation, unbound reactants were removed by extensive dialysis and protein concentration was determined by Lowry's method. The extent of protein glycation in the absence and presence of EA was evaluated by monitoring sub unit profile on SDS-PAGE, AGE related non-tryptophan fluorescence, protein carbonyl content and immunoblotting as described previously. The percentage of inhibition with EA was determined, considering the extent of glycation in the absence of the compound as 100%.

**MALDI-TOF/TOF analysis:** Prior to digestion, 50 µL of control and glycated lysozyme (in absence or presence of EA) aliquots were precipitated with 10-fold excess of acetonitrile and the dried precipitates were dissolved in 0.1 mL of 100 mM ammonium bicarbonate buffer (pH 8.5). The free thiol groups were reduced by the addition of 1 µL of 200 mM DTT (boiled for 5 min and incubated at RT for 30 min) followed by addition of 4 µL of 1 M iodoacetamide to alkylate the free cysteine groups. The solutions were incubated in the dark for 30 min at room temperature and the excess iodoacetamide was quenched in addition of 4 µL of 1.0 M DTT. Then the sample was dialyzed twice for 4 hr at room temperature against two 500 ml portions of water, followed by dialysis against 500 mL of 100 mM ammonium bicarbonate buffer (pH 8.2) for an additional 4 hr at room temperature. Protein (250 µg) was digested with 5 µg of the trypsin at 37°C for 18 h. The digested peptides were purified using C-18 zip tips (Millipore). The peptides eluted in 50% acetonitrile and 0.1% TFA were mixed with equal volume of cyano-4-hydroxycinnamic (10 mg/mL in 70% (v/v) acetonitrile, 0.1% (v/v) TFA). An aliquot (1 µL) of this mixture was spotted onto a stainless steel grid, air-dried, and subjected to mass determination using MALDI-ToF/ToF-MS (ABI 5800). The instrument was equipped with a nitrogen laser and operated in linear mode and in a positive-ion

delayed extraction reflector mode. Usually, each digest was spotted on at least three individual target positions and 250 individual spectra of each spot were averaged to produce a mass spectrum. Under these conditions, only single charged states were detected. The monitored mass range was  $m/z$  600-3500.

**Hemoglobin- $\delta$ -gluconolactone assay:** The assay involves the determination of glycated Hb (HbA<sub>1c</sub>) after incubation with  $\delta$ -gluconolactone using ion-exchange chromatography method. This method is specific for investigation of inhibitors on early glycation products (Amadori) formation. Samples were prepared by mixing 200  $\mu$ L of whole blood (collected from healthy human volunteers after overnight fast) with 50 mM  $\delta$ -gluconolactone in PBS in the absence and presence of varying concentrations of EA. Reaction contents were incubated at 37°C for 22 hr with occasional brief vortex-mixing. Blood samples were analyzed in duplicates for the determination of percentage of HbA<sub>1c</sub> using an ion-exchange system as per the manufacturer's protocol (Bio-systems).

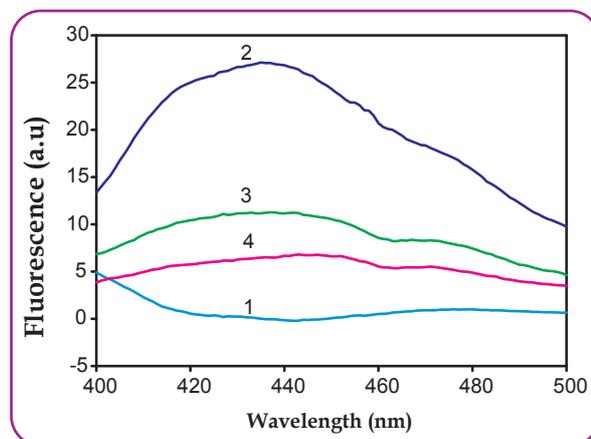
**Eye lens organ culture studies:** Three months old male Wistar rat lenses were cultured in modified M-199 medium containing 5 mM MGO in the absence and presence of 200  $\mu$ M EA when present for 3-5 days as described by us previously. After incubation, lenses were homogenized in 20 mM sodium phosphate buffer. The soluble fraction was separated from insoluble protein by centrifugation at 15,000 $\times$ g and protein concentration was estimated.

**Statistical Analysis:** Results were expressed as mean  $\pm$  SE. Data were analyzed using SPSS version 15.0 software. Mean values were compared by one-way analysis of variance (ANOVA) with post hoc tests of least significant difference method. Heterogeneity of variance was tested by non-parametric Mann-Whitney test. Differences between comparisons of groups were considered to be significant at  $P < 0.05$ .

## RESULTS

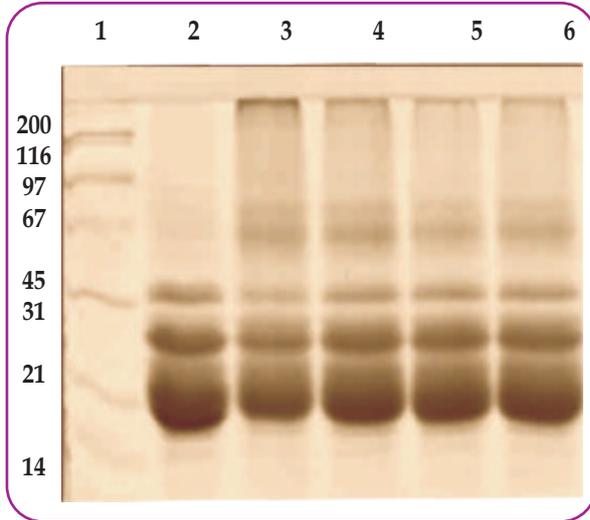
- EA showed dose-dependent inhibition of protein glycation and the response varied with the protein and the method used for the detection of glycation. In case of eye lens TSP, EA decreased AGE-fluorescence by about 50 and 90% at the concentrations of 50 and 100  $\mu$ M, respectively (Fig 30). EA inhibited the formation HMW aggregates upon fructation of TSP (Fig 31). Glyco-oxidative damage to TSP was also prevented by EA as there was a 50-90% reduction in the formation of protein-carbonyls upon glycation in presence of 100-150  $\mu$ M concentration (Fig 32).
- In order to highlight the antiglycating potential of EA, we have used *in vitro* glycation assays with three other proteins (Hb, lysozyme and BSA). In Hb glycation assay, while fructose reacts with Hb and forms cross-linked tetramers and HMW aggregates above 200 kDa, EA decreased HMW aggregates and recovery of native Hb (Fig 33A). In lysozyme glycation assay, lysozyme reacts with ribose and forms glycated lysozyme dimer and tetramers. In presence of increasing

**Fig 30. Representative non-tryptophan AGE fluorescence of eye lens TSP upon *in vitro* glycation in the absence and presence of EA.** Trace 1, protein alone; trace 2, protein + 200 mM fructose; trace 3, protein + fructose + 50  $\mu$ M EA; trace 4, protein + fructose + 100  $\mu$ M EA. a.u, arbitrary units.



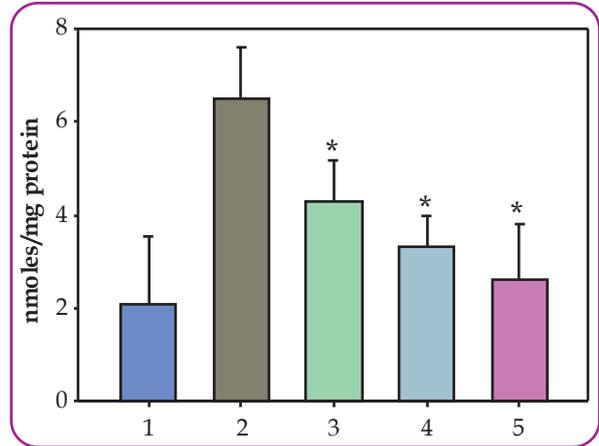
**Fig 31. Representative SDS-PAGE profile of eye lens TSP upon *in vitro* glycation in the absence and presence of EA.**

Lane 1, molecular weight marker; lane 2, protein alone; lane 3, protein+200 mM fructose; lane 4, protein+ fructose +50  $\mu$ M EA; lane 5, protein+fructose+100  $\mu$ M EA; lane 6, protein+fructose+150  $\mu$ MEA



**Fig 32. Protein carbonyl content of soluble lens protein upon *in vitro* glycation in the absence and presence of EA.**

Bar 1, protein alone; bar 2, protein+200 mM fructose, bar 3, protein+fructose+50  $\mu$ M EA; bar 4, protein+fructose+100  $\mu$ M EA, bar 5, protein+fructose+150  $\mu$ MEA.



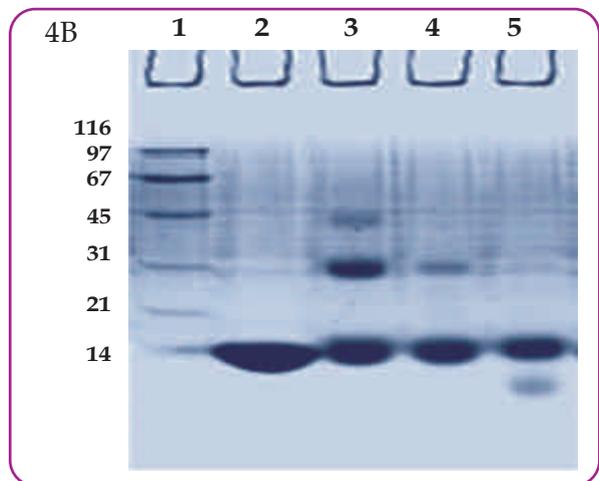
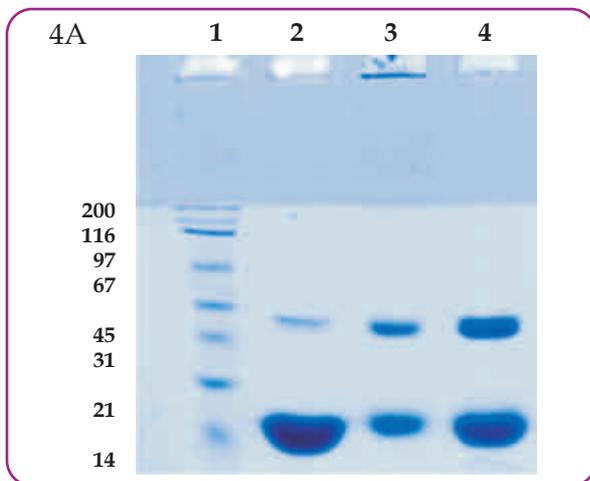
Data are mean $\pm$ SE of three independent experiments. \*Mean values were significantly different from that of bar 2 ( $P < 0.05$ )

concentration of EA, disappearance of the dimer and tetramer bands of lysozyme was noted as monitored by SDS-PAGE (Fig 33B). Similarly, EA also inhibited the AGE formation on BSA (data not shown).

- AGE is a collective term referred to a heterogeneous group of chemical structures that range from CML to more complex structures such as pentosidine or vesperlysine. Hence, the ability of EA to inhibit some of these AGE by immunodetection was investigated. Data obtained with immunoblotting demonstrated inhibition of CML and AGE by EA (Fig 34).

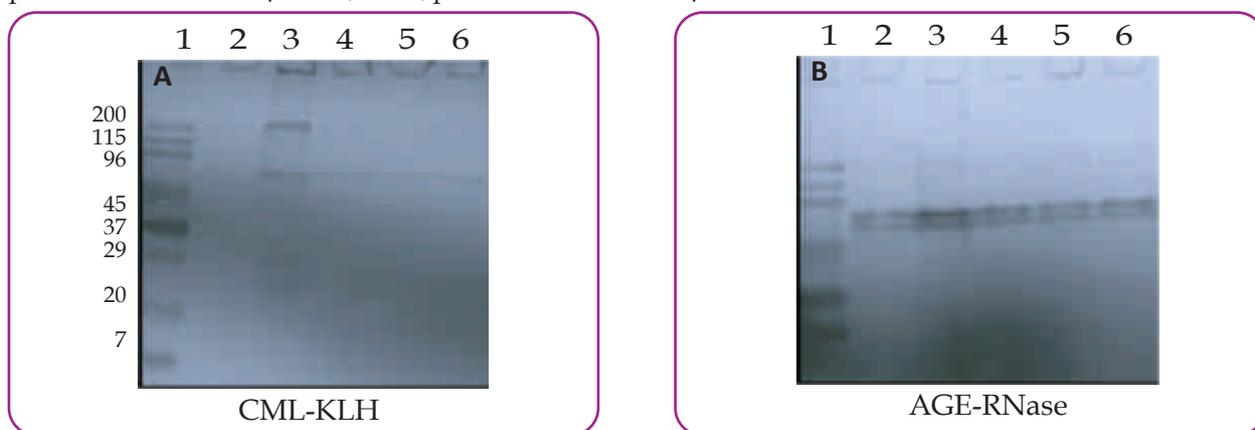
**Fig 33. Representative SDS-PAGE profile of human Hb (Panel A) and lysozyme (Panel B) upon *in vitro* glycation in the absence and presence of EA.**

Lane 1, molecular weight marker; lane 2, protein alone; lane 3, protein+200 mM fructose; lane 4, protein+fructose+100  $\mu$ M EA, lane 5, protein +fructose+200  $\mu$ MEA.



**Fig 34. Immunodetection of AGE in eye lens TSP**

Representative western blots of eye lens TSP upon *in vitro* glycation in the absence and presence of EA. Blots were probed with anti-CML-KLH (A) and AGE-RNase (B) antibodies. Lane 1, molecular weight markers; lane 2, protein alone; lane 3, protein+200 mM fructose; lane 4, protein+fructose+50  $\mu$ M EA; lane 5, protein+fructose+100  $\mu$ M EA; lane 6, protein+fructose+200  $\mu$ M EA.



- Inhibition of CML or CEL by EA was further confirmed by MALDI-ToF/ToF. For this we have glycated the lysozyme with MGO. MALDI-ToF-MS of intact lysozyme produced symmetric singly charged mass peak at 14313 amu. However, peak broadening with several unresolved antenna peaks appeared in the mass spectrum of glycated lysozyme. Although, peak broadening has disappeared in the presence of EA and its effects on specific modifications could not be identified in linear mode. This necessitated the protein digestion prior to the analysis.
- The MALDI-ToF/ToF peptide mass spectrum of lysozyme incubated with MGO in the absence and presence of EA was shown in (Fig 35). The peptide masses observed in the mass spectrum were in complete agreement with that of the theoretical peptide masses generated in silico (MS-digest). However, two additional mass peptide peaks (3080 and 3216 amu) were observed in the glycated lysozyme (Fig 35A & 35B), which are likely modification of peptides 3008 amu and 3163 amu, respectively present in the native lysozyme due to formation of CEL (molecular mass 72 amu) (Fig 35A) and hydroimidazolone (molecular mass 54 amu) (Fig 35B and Table 29). Interestingly, presence of EA (50 and 200  $\mu$ M) inhibited the CEL and hydroimidazolone modification as indicated by the decreased abundance of the 3080 and 3216 amu mass peaks, as indicated by the decrease in area under the curve of mass peaks. MS digest analysis suggests that the CEL modification site lies between amino acids 87-114 while the

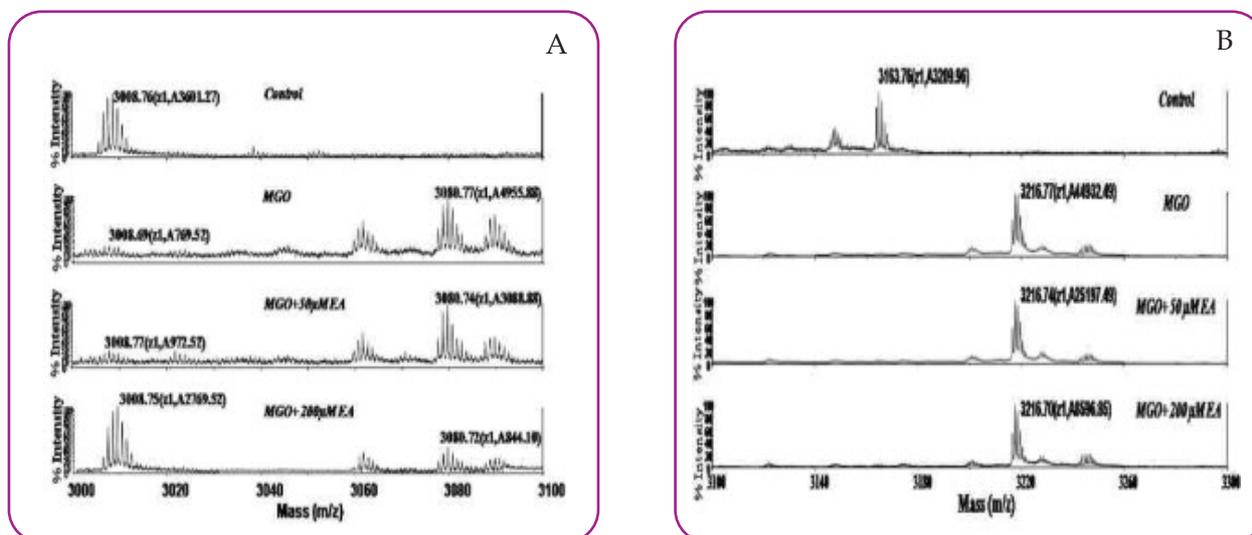
**Table 29. Identification of molecular location of MGO modification products in lysozyme**

Protein	Observed ion	Theoretical peptide mass*	Peptide sequence*	Mass increase	Modification	Glycated residue
Lysozyme	3080.76	3008.77	87RTPGSRNLCNIPC SALLSS DITASVNC AKK114	72	CEL	K113
	3216.70	3163.76	52KFESNFNTQATN RNTDGSTDY GILQI NSRW79	53	Hydroimidazolone	R64 or R78

\* The theoretical peptide masses and corresponding sequences were obtained from the *in silico* digestion of lysozyme sequence (Pubmed accession No: 11788708) using the MS Digest software (<http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msdigest>).

**Fig 35. MALDI-ToF MS analysis of lysozyme glycation in the absence and presence of EA.**

MALDI-ToF mass spectra of digested lysozyme control or lysozyme incubated with 5 mM MGO in presence of EA (50  $\mu$ M, 200  $\mu$ M) were obtained. The representative MALDI-MS spectra of lysozyme glycated peptides were shown in truncated spectra. CEL modification in the range of 3000-3100 amu (A) hydroimidazolone modification in the range of 3100 to 3300 amu (B) were shown. The ionization state (z) and the peak area were also given for respective mass peaks.



hydroimidazolone modification lies between 52-79 amino acids, respectively. The peptide (87-114) composes of the two Lys residues at positions 113 and 114. Modification of Lys 113 by MGO is therefore highly likely, as modification of Lys 114 would have eliminated the tryptic digestion site. The amino acid sequence corresponding to 52-79 amino acids composes of two Arg residues at positions 64 and 78 therefore, one of them could have been modified by MGO. Together these results suggest that EA efficiently inhibits the MGO induced CEL and hydroimidazolone modifications of lysozyme.

6. Eventually, the significance of antiglycating potential of EA was assessed using two different *ex vivo* models, which mimic the *in vivo* conditions. In the first model, the ability of EA was determined by estimating the amount of formation of HbA<sub>1c</sub> in whole blood under *ex vivo* conditions. Human RBC incubated with  $\delta$ -gluconolactone showed high levels of HbA<sub>1c</sub> as compared with control RBC. Interestingly, EA inhibited the HbA<sub>1c</sub> formation in RBC under these conditions (Table 30).
7. In order to confirm inhibition of AGE formation and to endorse the utility of EA, eye lens organ culture model was employed. Incubation of rat lens with 5 mM MGO resulted in loss of

**Table 30. Inhibition of formation of HbA<sub>1c</sub> in human blood incubated with  $\alpha$ -gluconolactone under *ex vivo* conditions in absence and presence of EA.**

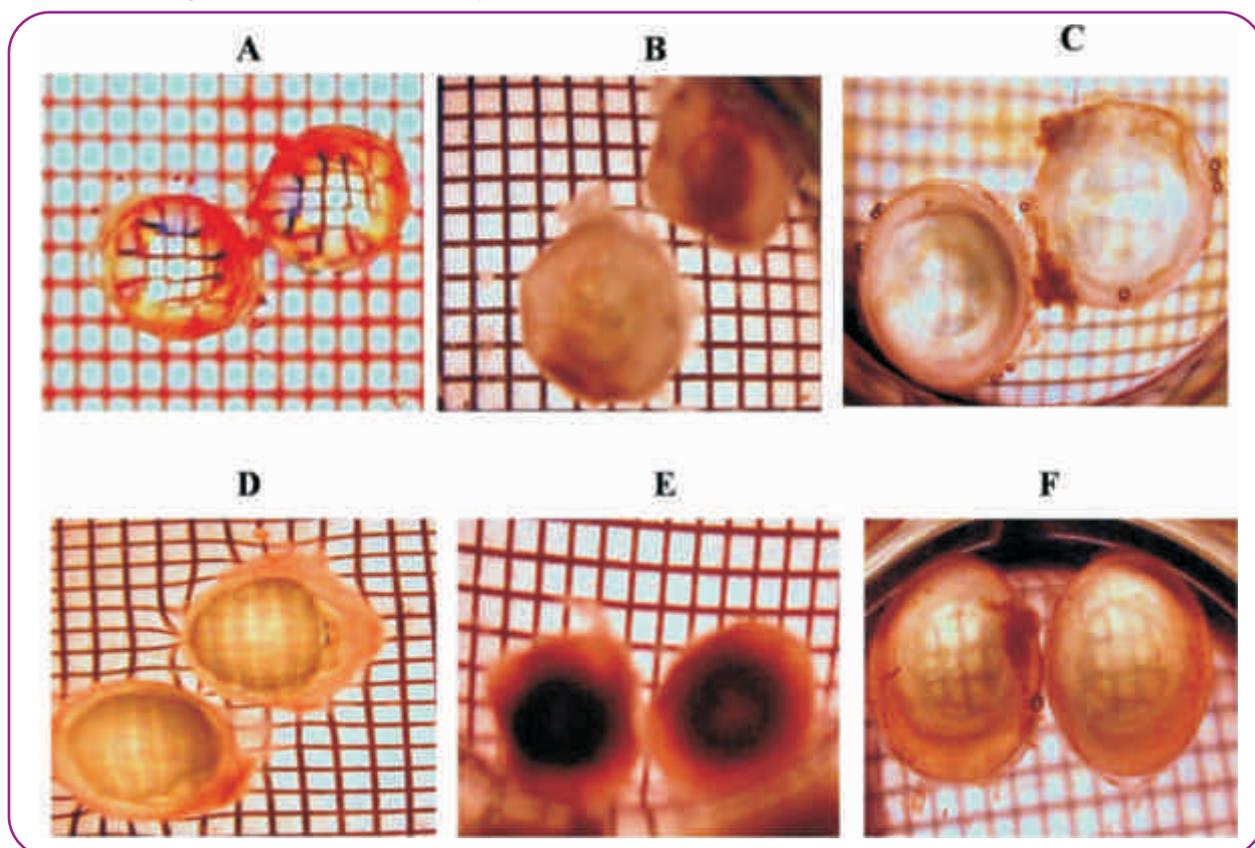
Conditions	HbA <sub>1c</sub> (%)
Control blood	5.73 $\pm$ 0.607
Blood + $\delta$ -gluconolactone	8.87 $\pm$ 0.506
Blood + $\delta$ -gluconolactone + 100 $\mu$ M EA	7.23 $\pm$ 0.207*
Blood + $\delta$ -gluconolactone + 500 $\mu$ M EA	6.49 $\pm$ 0.400*

The percentage of HbA<sub>1c</sub> was determined using an ion-exchange system in duplicates. Data are mean SE (n=3). \*Mean values were significantly different from that of blood with  $\delta$ -gluconolactone in the absence of EA (P<0.05)

transparency (opacification) in a time-dependent manner as assessed by the transmission of the light from the grid through the lens (Fig 36). Interestingly, EA prevents the loss of transparency.

**Fig 36. Effect of EA on MGO-induced opacification in lens organ culture studies.**

Rat lenses were cultured in modified M-199 medium as described in methods. Lens in the medium alone (A), in the presence of 5 mM MGO for 3 days (B), in the presence 5 mM MGO along with 200  $\mu$ M EA for 3 days (C), lens in the medium alone (D) for 5 days, in the presence of 5 mM MGO for 5 days (E), and in the presence 5 mM MGO along with 200  $\mu$ M EA for 5 days (F).



## CONCLUSIONS

A new template of antiglycating compound in the form of EA described structurally and provided mechanistic explanation of its action. Herein, for the first time, the potential of EA to inhibit or prevent the formation of AGE on different model proteins and its possible mechanism and importance of action was demonstrated. Inhibition of AGE formation by EA was demonstrated with model proteins such as TSP, Hb, lysozyme and bovine serum albumin using various commonly used glycating agents employing an array of complementary methods. These results suggest that, antiglycating action of EA seems to involve apart from the inhibition of a few fluorescent AGE, predominantly inhibition of CML. Further, prevention of loss of eye lens transparency through inhibition of AGE supports the antiglycating potential of EA *in vivo*. Noteworthy finding was that EA also inhibited the HbA<sub>1c</sub> formation in human RBC under conditions that mimic the situation of HbA<sub>1c</sub> formation *in vivo*. These findings are indicated that the potential of EA to prevent and/or inhibit protein glycation. Since, AGE were the main protagonists of diabetic complications, the antiglycating ability and the potential to ameliorate the diabetic complications makes EA a suitable compound for the treatment of AGE-associated pathologies.

## Characterization of active principles and mechanism of action of dietary aldose reductase inhibitors and antiglycating agents: (IV) Inhibition of advanced glycation end product formation on eye lens protein by rutin and ellagic acid

Long-term secondary complications of diabetes represent the main cause of morbidity and mortality in diabetic patients. Although mechanisms leading to diabetic complications are not completely understood, many biochemical pathways associated with hyperglycemia have been implicated. Among these, polyol pathway and advanced glycation end-products (AGE) formation have been extensively studied. Glycation is a non-enzymatic reaction between reducing sugar and free amino group of the protein forming an Amadori product. The latter then undergoes a series of complex reactions (oxidative and nonoxidative) resulting in the formation of AGE. Formation of AGE plays a key role in the development of several pathophysiological processes associated with aging and diabetes such as atherosclerosis, chronic renal insufficiency, nephropathy, neuropathy and cataract. This raises the possibility that inhibition of AGE formation may prevent the progression of diabetic complications. However, designing a drug having anti-AGE activity is a challenge due to the complexity of reactions involved in the formation of AGE.

A wide variety of agents like pyridoxamine, carnosine, phenyl thiazolium bromides, aspirin, and lipoic acid have been investigated in several *in vitro* and *in vivo* studies for their potential against various pathologies including cataract. However, except pyridoxamine, none have passed all the stages of clinical trials. While the extensively investigated hydrazine compound, aminoguanidine, has shown promising results *in vitro* and in animal models in terms of inhibition of AGE-formation and entered into phase 3 clinical trials the trial was terminated due to various safety concerns. It is known that glycation and AGE-induced toxicity are associated with increased free radical activity. Recent studies have demonstrated the benefits of using compounds with combined antiglycation and antioxidant properties. Such compounds not only prevent AGE formation but also reduce free radical-mediated toxicity. Hence, efforts are being made in identifying natural sources of antiglycating agents that can be tested for their therapeutic value against AGE-mediated pathologies.

In the course of identifying and testing new antiglycating agents, a number of traditional and very common dietary sources were evaluated and found that some spice principles, fruits and vegetable have the potential to inhibit AGE formation under *in vitro* conditions and in animal models. Flavonoids are abundantly found in fruits, vegetables, herbs and spices, and some of the flavonoids have been tested for their antiglycating activity. Rutin is one of the commonly found dietary flavonols. Therefore, in this study the effect of rutin against glycation-induced alterations of lens protein was investigated. A set of complementary methods were employed; spectroscopic, electrophoretic, chromatographic and immunochemical, to evaluate antiglycating potential of rutin as well as its mechanism of action.

### Experimental

***In vitro glycation of proteins and inhibition with rutin:*** Each 1 mL reaction mixture contained 10mg protein (total soluble protein, TSP from eye lens or BSA) in 0.2 M phosphate buffer, (pH 7.4), 0.2 M fructose, 50 µg of penicillin and streptomycin and 0.01% sodium azide. For inhibition studies, various concentrations of rutin (10-1000 µM) were added to *in vitro* protein glycation assay mixture and incubated in dark at 37° C for 1-3 weeks as described above. At the end of the incubation, unbound reactants were removed by extensive dialysis and protein concentration was determined by Lowry's method. The extent of protein glycation in the absence and presence of rutin was

evaluated by monitoring sub unit profile on SDS-PAGE, AGE related non-tryptophan fluorescence, protein carbonyl content and immunoblotting as described previously. The percent of inhibition with rutin was determined, considering the extent of glycation in the absence of the compound as 100%.

**Metal Chelation:** Metal chelating activity was assessed by determining the metal-induced oxidation of ascorbic acid to dihydroascorbic acid using HPLC method as described previously. Copper chloride (1 $\mu$ M) was preincubated with and without rutin in Chelex-100 treated Milli-Q element free water for 5 min, and then the ascorbate (0.01 mg/mL) was added to the reaction mixture to initiate the metal-catalyzed oxidation reaction. Oxidation of ascorbic acid was analyzed by HPLC.

**Spectral absorbance shift:** Shift in absorbance spectrum of rutin due to complex formation with CuCl<sub>2</sub> was recorded. Absorbance of rutin in the absence and presence of 100  $\mu$ M CuCl<sub>2</sub> in Chelex-100 treated 50 mM sodium phosphate buffer, (pH 7.4), was scanned from 200-700 nm in spectrophotometer.

**Post-Amadori inhibition:** BSA (10 mg/mL) was incubated with ribose (0.4 M) at 37°C in 0.4 M phosphate buffer, pH 7.4, for 24 h. Glycation was then interrupted to remove excess ribose and also reversible Schiff base by extensive dialysis against 20 mM sodium phosphate buffer at 4°C. The glycated BSA intermediate containing maximal amount of Amadori product was then incubated at 37°C in the absence or presence of rutin, for 5 days. This initiated conversion of Amadori intermediates to AGE products and the extent of conversion of Amadori to AGE was measured by non-tryptophan AGE fluorescence.

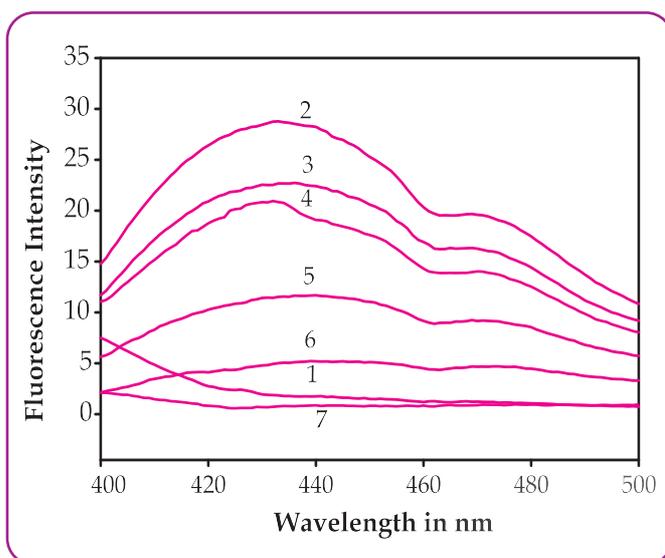
**Statistical Analysis:** Results were expressed as mean  $\pm$  SE. Mean values were compared by one-way analysis of variance (ANOVA) with post hoc tests of least significant difference method. Heterogeneity of variance was tested by non-parametric Mann-Whitney test. Differences between comparisons of groups were considered to be significant at P<0.05.

## RESULTS

1. Rutin inhibited AGE-related fluorescence in a dose dependent manner with 90% of reduction in AGE-fluorescence at 200  $\mu$ M (Fig 37).
2. Incubation of lens protein with fructose led to the appearance of non-disulfide dimers with a molecular weight of approx 45 kDa and a large amount of HMW aggregates above 200 kDa that did not enter the stacking gel was observed on the SDS-PAGE (Fig 38), similar to the modifications observed in the soluble portion of lens protein from STZ-induced diabetic cataract. Rutin at 100  $\mu$ M was found to reduce the formation of both cross-link and HMW aggregates (Fig 38).

**Fig 37. Inhibition of AGE formation by rutin**

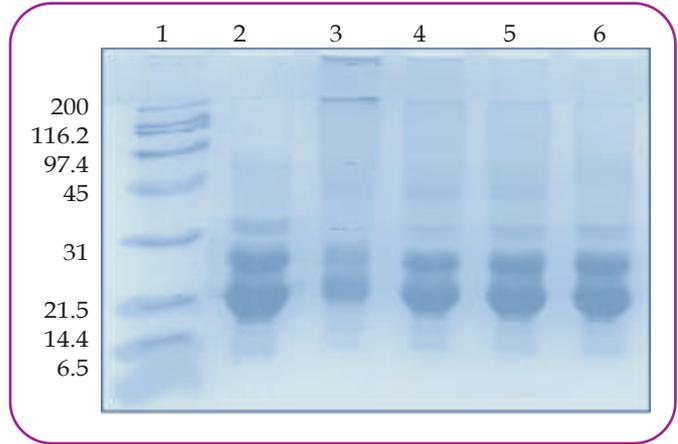
Representative non-tryptophan AGE related fluorescence of TSP upon in vitro glycation in the absence and presence of rutin. Trace 1- protein alone (P), trace 2 - P + 100 mM Fructose (F), trace 3 - P + F + 10  $\mu$ M rutin, trace 4 - P + F + 50  $\mu$ M rutin, trace 5 - P + F + 100  $\mu$ M rutin, trace 6 - P + F + 200  $\mu$ M rutin, trace 7 - P + 200  $\mu$ M rutin.



3. Immunodetection with anti-AGE antibodies demonstrated the presence of diverse antigenic determinants over the protein (Fig 39A). Anti-MGO-BSA detected cross-linked species of 45 and 26 kDa along with HMW aggregates >118 kDa. Anti-CML-KLH detected the HMW aggregates >118 kDa along with intermediate species of 45 and 26 kDa and anti-AGE-RNase detected the intermediate cross-linked species of 85 kDa. Densitometry analysis (Fig 39B) indicates that CML and MGO derived AGE were prominent than glucose derived AGE. Densitometry analysis showed that rutin at 50  $\mu$ M could reduce CML derived AGE by approximately 90%, glucose-derived AGE by 60%, and 90% reduction for the MGO derived modifications, respectively.

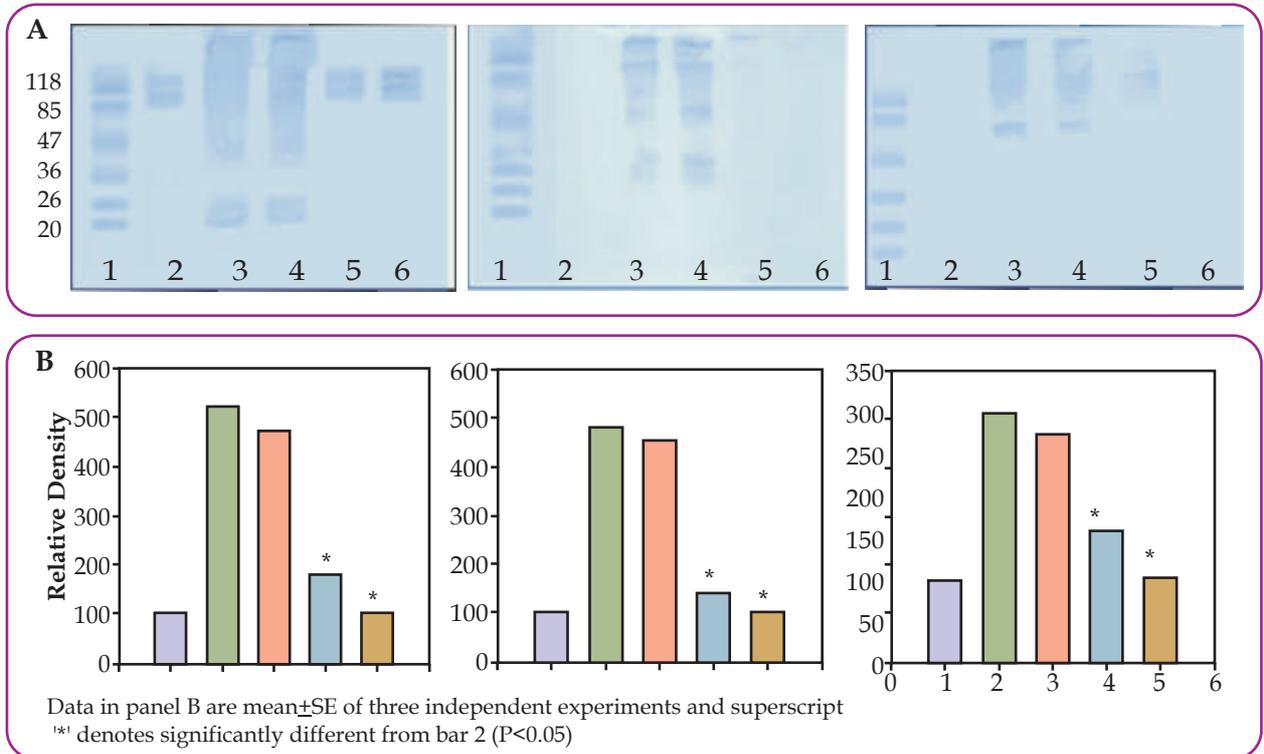
**Fig 38. Inhibition of AGE mediated protein cross-links by rutin**

Representative SDS-PAGE profile of TSP upon in vitro glycation in the absence and presence of rutin. Lane 1- molecular weight markers, lane 2- protein alone (P), lane 3 - P + 100 mM Fructose (F), lane 4 - P + F + 10  $\mu$ M rutin, lane 5 - P + F + 50  $\mu$ M rutin, lane 6 - P + F + 100  $\mu$ M rutin.



**Fig 39. Immunodetection of AGE in soluble lens protein**

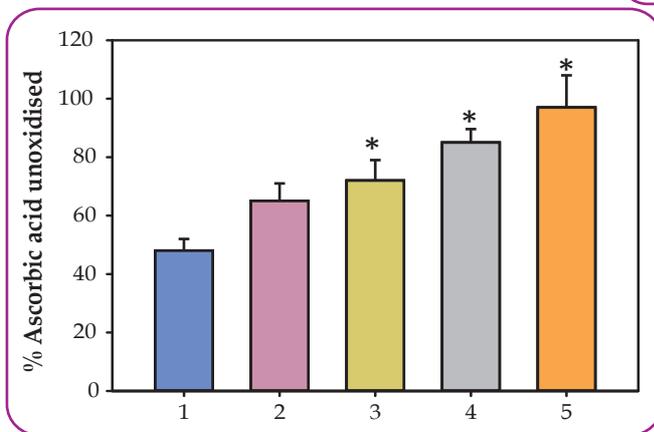
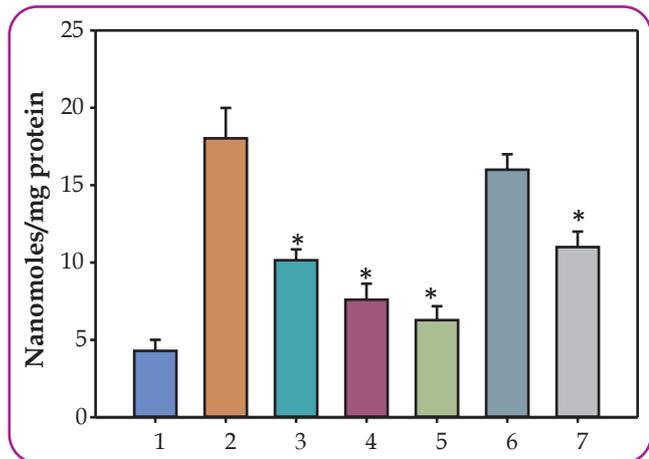
Panel A: Representative western blot profile of TSP upon in vitro glycation in the absence and presence of rutin. Blots were probed with anti-MGO-BSA (top), anti-CML-KLH (middle) and anti-AGE-RNase antibodies (bottom). Lane 1 - molecular weight markers, lane 2 - protein alone (P), lane 3 - P + 100 mM fructose (F), lane 4 - P + F + 10  $\mu$ M rutin, lane 5 - P + F + 50  $\mu$ M rutin and lane 6 - P + F + 100  $\mu$ M rutin. Panel B: Densitometry analysis of AGE. Intensity of AGE signals was quantified considering the intensity of lane 2 (in panel A) as 100 %. Bars 1-5 in panel B correspond to lanes 2-6 of panel A.



4. Increased carbonyl content (four fold) of lens proteins upon fructose modification is an indicator of glycooxidative damage (Fig 40).
5. Reduction of  $\text{CuCl}_2$ -catalyzed oxidation of ascorbic acid by rutin in a dose dependent manner (Fig 41) indicates that inhibition of AGE by rutin could be due its metal chelation ability. Further, a shift in the absorbance spectrum of rutin in the presence of  $\text{CuCl}_2$  confirms formation of rutin-metal complex (Fig 42) and supports that rutin may have metal chelation property.
6. Finally, we have also assessed the potential of rutin to inhibit post-Amadori reaction as Amadori product after several rearrangement leads to formation of stable and heterogeneous AGE. Rutin partly (30%) inhibited the post-Amadori compound formation at 100  $\mu\text{M}$  concentration which is comparable to that of aminoguanidine at 10 mM (Fig 43).

**Fig 40. Protein carbonyl content of TSP upon in vitro glycation in the absence and presence of rutin.**

Bar 1- protein alone (P), bar 2 - P + 100 mM Fructose (F), bar 3 - P + F + 10  $\mu\text{M}$  rutin, bar 4 - P + F + 50  $\mu\text{M}$  rutin bar 5 - P + F + 100  $\mu\text{M}$  rutin, bar 6 - P + F + 10 mM aminoguanidine, bar 7-P+F+100mM aminoguanidine. Data are mean SE of three independent experiments and super-script<sup>†\*</sup> denotes significantly different from bar 2 (P<0.05)

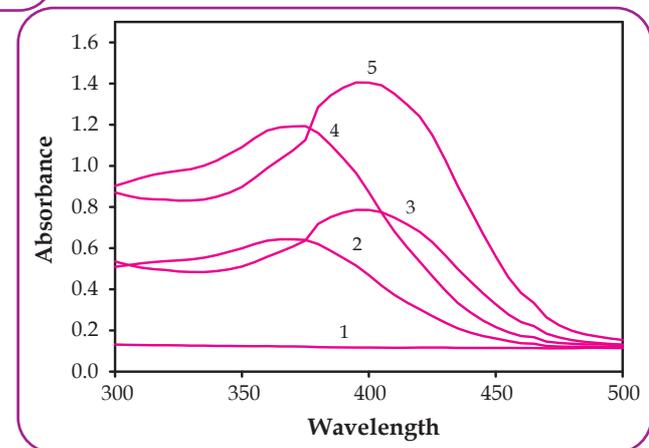


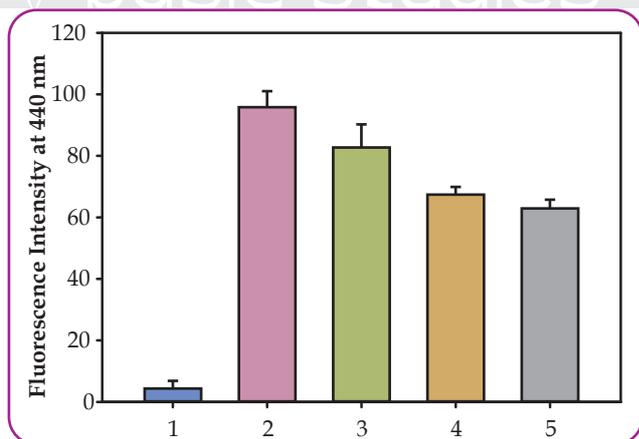
**Fig 41. Chelation of metals by rutin**

Percentage ascorbic acid un-oxidized due to metal catalysed reaction in the absence and presence of rutin. Bar 1- Ascorbic acid (AA) +  $\text{CuCl}_2$ , bar 2- AA +  $\text{CuCl}_2$  + 50  $\mu\text{M}$  rutin, bar 3- AA+ $\text{CuCl}_2$  + 100  $\mu\text{M}$  rutin, bar 4- AA +  $\text{CuCl}_2$  + 500  $\mu\text{M}$  rutin, bar 5- AA+  $\text{CuCl}_2$ + 1000  $\mu\text{M}$  rutin. Data are mean $\pm$ SE of three independent experiments and superscript <sup>†\*</sup> denotes significantly different from bar 1 (P<0.05).

**Fig 42. Spectral shift of rutin in the presence of  $\text{CuCl}_2$**

Absorption spectrum of 50  $\mu\text{M}$  rutin in the absence (trace 2) and presence of 1  $\mu\text{M}$   $\text{CuCl}_2$  (trace 3), absorption spectrum of 100  $\mu\text{M}$  rutin in the absence (trace 4) and presence of 1  $\mu\text{M}$   $\text{CuCl}_2$  (trace 5). Absorption spectrum of 1  $\mu\text{M}$   $\text{CuCl}_2$  alone is also recorded (trace 1).





**Fig 43. Inhibition of post-Amadori product formation by rutin**

AGE fluorescence of BSA upon incubation with 0.4 M ribose in the absence and presence of rutin was recorded at 440 nm upon excitation at 370 nm. Bars 1-5 correspond to BSA alone, BSA + ribose, BSA + ribose + 50 μM rutin, BSA + ribose (R) + 100 μM rutin and BSA + ribose (R) + 200 μM rutin, respectively. Data are mean±SE of three independent experiments and superscript <sup>1\*</sup> denotes significantly different from bar 2 (P<0.05).

## CONCLUSIONS

In this study, the antiglycating effect of rutin was demonstrated. Further, insight into the mechanism of inhibition of protein glycation that rutin not only scavenges free radicals directly but also chelates the metal ions by forming complexes with them and partly inhibits post-Amadori formation was provided. These findings indicate the potential of rutin to prevent and/or inhibit protein glycation and prospects for controlling AGE-mediated diabetic pathological conditions *in vivo*. Studies are underway to investigate the potential of rutin against STZ-induced diabetic complications in animal models.

## 13 Characterization of retinal degeneration in an obese rat model: Amelioration of retinal degeneration in WNIN/Ob rat by vitamin A supplementation

Obesity is a major public health problem worldwide, and of late, epidemiological studies indicate a preponderance of ocular problems under obesity conditions. The ocular complications of obesity include diabetic retinopathy, high intraocular pressure, cataracts, macular degeneration and exophthalmos. Evidence is accumulating to show that obesity is associated with increased risk of retinal (macular) degeneration and diabetic retinopathy. Obese men are more than twice as likely to have dry macular degeneration as men with a normal basal metabolic index. An association between higher BMI and early macular degeneration has been reported. It is reported that abdominal obesity in patients with early or intermediate stages of macular degeneration increases the risk for progression to AMD. However, experimental basis for obesity associated retinal degeneration has not been established yet. In this regard, retinal degeneration in a spontaneously developed novel obese rat model was observed. In this study WNIN/Ob rats develop progressive retinal degeneration, particularly rod cell loss after the onset of severe obesity was demonstrated. The WNIN/Ob rat is the first rat model to develop retinal degeneration after the onset of obesity, mainly due to impaired phototransduction, and photoreceptor degeneration. Thus this novel rat model may be a valuable tool for investigating retinal degeneration associated with obesity, particularly intervention studies.

Vitamin A, an important micronutrient, has an unusually wide range of vital biological functions in the mammalian system. The visual cycle is the system in which the role of vitamin A is thoroughly understood. It has been firmly established that the vitamin A is required for vision in dark and also for colour perception. Because of its utmost importance in the visual cycle, vitamin A had been extensively used in the treatment of many retinal diseases. For example, in a randomized clinical trial, retinitis pigmentosa (RP) patients who received oral vitamin A supplementation showed slower decline in the cone response compared to patients who received either vitamin E or no vitamin supplementation. Vitamin A supplementation slows the rate of photoreceptor degeneration caused by a class II rhodopsin mutation. Interestingly, earlier it was shown that chronic dietary vitamin A supplementation at high doses was effective in regulating obesity in WNIN/Ob rat, possibly through up-regulation of the brown adipose tissue (BAT)- uncoupling protein-1 (UCP1) gene and associated adipose tissue loss. It was also shown that vitamin A decreases body weight gain in WNIN/Ob rat independent of stearoyl-CoA desaturase-1 (SCD1) gene regulation. Hence, in the present study the effect of vitamin A supplementation on retinal degeneration in WNIN/Ob rat by evaluating morphology, gene and protein expression in the retina of WNIN/Ob rat was investigated.

### Experimental

**Animal study design and dietary regimen:** Five-month-old male lean (L; n=32) and obese (O; n=32) phenotype of WNIN/Ob rats were subdivided into four groups (L/O-I, L/O-II, L/O-III and L/O-IV). Subgroups LI and OI received the stock diet, which provided 2.6 mg of vitamin A/ kg diet and served as control groups. Subgroups L/O-II, L/O-III and L/O-IV received 26, 52, and 129 mg of vitamin A/kg diet as retinyl palmitate respectively. All diets were identical with regard to all other ingredients except the vitamin A content. The dosage of vitamin A was decided based on previous studies wherein, optimal total intake of vitamin A was approximately 18,000 IU per day for treating retinal pathologies and doses of 25,000 IU or more over the long term are considered potentially toxic. In the present study, the highest dosage of vitamin A i.e, 129 mg/ kg diet corresponds to 10,500 IU only according to daily intake of obese rat. All procedures involving rats were performed in accordance and with approval by the Institutional Animal Ethics Committee. Animals were fed on their respective diets for a period of four and half months. At the end of the study, rats were sacrificed by CO<sub>2</sub> asphyxiation after 12 hr fasting. Retina was dissected from the eyeball and frozen in liquid nitrogen and stored at -80°C until further analysis.

**RNA extraction and quantitative real-time PCR:** Total RNA was isolated from retinal tissue using TRI reagent and reverse transcription was performed with total RNA using cDNA synthesis kit. Quantitative real-time PCR was performed using cDNA of each sample in triplicates in a reaction mixture consisting of SYBR Green supermix, forward and reverse primers of target gene with a thermocycler. The expression of genes involved in phototransduction like rhodopsin (*Rho*), phosphodiesterase (*Pde6b*), rod arrestin (*Sag*), rod, cone transducins (*Gnat1* & *Gnat2*) and elongation of very long chain fatty acids-4 (*Elovl4*, mutations in which is implicated in macular dystrophy) that were previously shown to be down-regulated in obese rat retina [14] was analysed by quantitative real-time PCR. The expression of stress response genes, glial fibrillary acid protein (*Gfap*) and vascular endothelial growth factor (*Vegf*) whose expression was shown to be up-regulated in obese rat retina was also analyzed. Hypoxanthine phosphoribosyltransferase (*Hgppt*) was also amplified from the samples and served as house keeping control. The target gene expression values were normalized to house keeping gene (*Hgppt*) according to comparative Ct-value method for relative quantification.

**Immunohistochemistry:** Eyeballs were collected from vitamin A supplemented lean and obese animals and fixed in paraformaldehyde in phosphate buffer (pH 7.2), followed by embedding and sectioning, as described previously. Immunohistochemistry was performed on retinal sections

using respective primary antibody, as described previously. The slides were mounted in antifade reagent and were observed under an epifluorescence microscope.

**Histopathology:** For histological study, eyeballs of experimental rats were dissected out and fixed in 10% neutral buffered formalin overnight and processed. The tissues were later paraffin embedded and blocks were prepared. Haematoxylin and eosin staining was done on 4  $\mu\text{m}$  paraffin sections and retinal morphology was examined under the microscope.

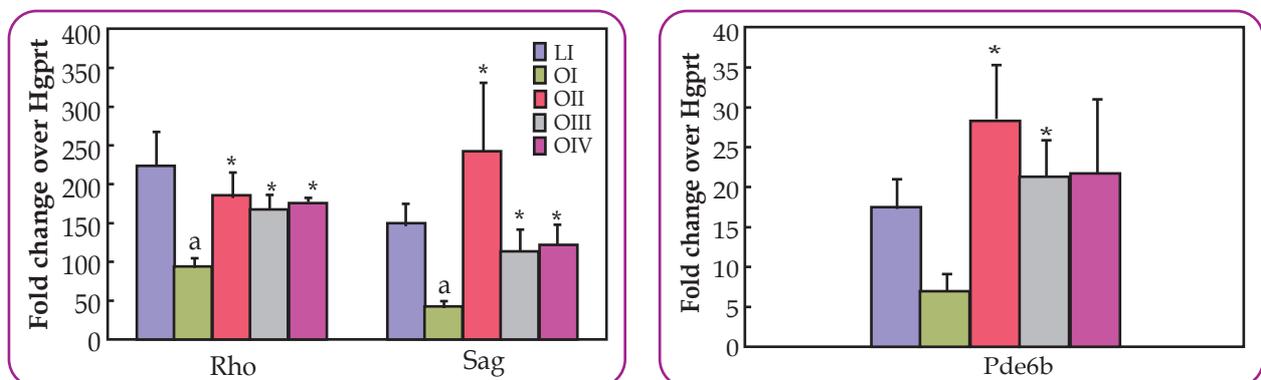
**Statistical analysis:** Results were expressed as mean values and standard errors. Data was log transformed. Mean values were compared by One-way ANOVA with *post hoc* tests of the least significant difference method. Differences between comparisons of groups were considered to be significant at  $p \leq 0.05$ .

## RESULTS

1. Pre-treatment body weights were comparable among the lean and among the obese groups of WNIN/Ob rats. While the body weights of lean rats were unaltered upon supplementation of various doses of vitamin A, the body weight of O-III and O-IV groups had decreased significantly when compared with the O-I group.
2. The plasma retinol was higher in obese groups when compared with the lean groups and vitamin A supplementation had no effect on plasma retinol levels.
3. There was no difference in the expression level of genes studied among all the lean groups by vitamin A supplementation. Previously, we reported that expression of many retinal structural and functional genes was decreased in WNIN/Ob rat retina when compared with its lean littermates, whereas expression of Gfap and Vegf was increased. Hence we considered only L-I as control and compared it with O-I for depicting the difference between lean and obese as these two groups were given a normal dosage of vitamin A (2.6 mg/kg diet). Whereas O-I group has been taken as an untreated obese group so as to understand the modulation of retinal gene expression in obese rat retina by vitamin A supplementation (i.e. in comparison with O-II, O-III, O-IV).
4. The decreased expression of rod specific genes: Rho, Sag, Gnat1 due to obesity was significantly modulated (increased) upon vitamin A supplementation in WNIN/Ob rats (Fig 44 & 45).

**Fig 44. Expression of Rho and Sag (Left Panel) and Pde6b (Right Panel) in the retina of WNIN/Ob rats.**

Expression values are determined by qRT-PCR and presented on an arbitrary scale after normalization with Hgprt.

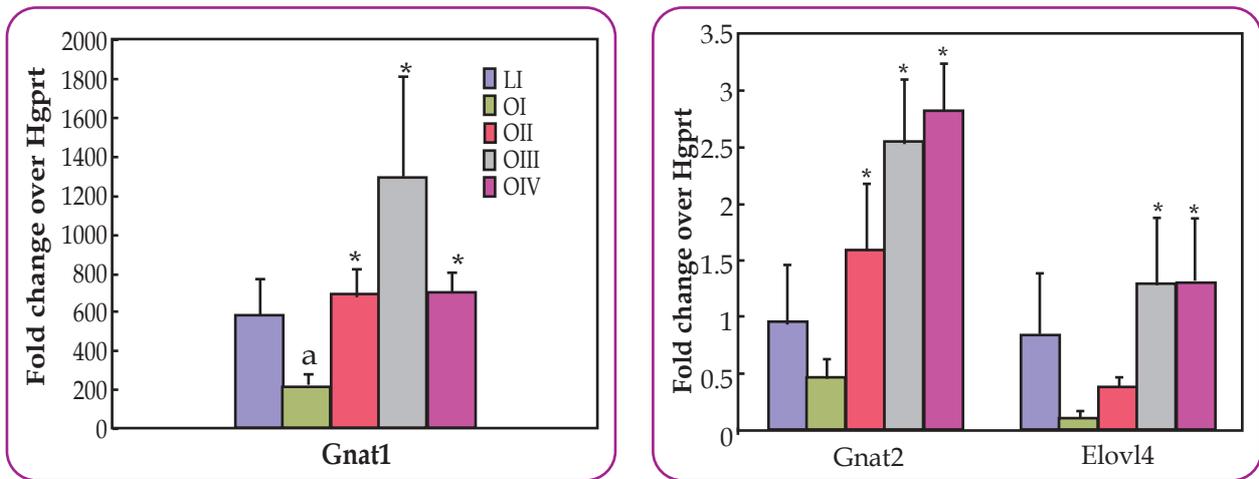


Data represent mean  $\pm$  SE (n=6). Bars with superscript “\*” and “a” are significantly different when compared with the group O-I and L-I, respectively at  $p \leq 0.05$  by One way ANOVA. L-lean, O-obese; L/O-I, O-II, O-III and O-IV are groups that received 2.6, 26, 52 or 129 mg of vitamin A / kg diet as retinyl palmitate respectively.

5. However, the extent of modulation of different genes varied with the dose of vitamin A. While the expression of Rho increased by 1 fold, expression of Sag increased by 4.5 fold and expression of Pde6b increased by 6 fold in O-II group (Fig 44), the expression of rod transducin Gnat1 increased by 4.7 fold in O-III group and that of cone transducin Gnat2 increased significantly by 2.13 fold in O-IV group (Fig 2) when compared with the O-I group. The expression of Elov14 had significantly increased by 2.5 folds in O-III and O-IV groups compared with the O-I group (Fig 45).

**Fig 45. Expression of Gnat1 (Left Panel) and Gnat2 and Elov14 (Right Panel) in the retina of WNIN/Ob rats**

Expression values are determined by qRT-PCR and presented on an arbitrary scale after normalization with *Hgprt*.

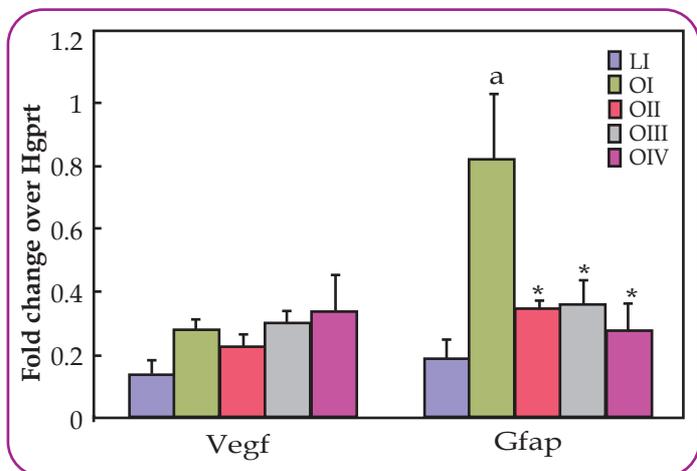


Data represent mean  $\pm$  SE (n=6). Bars with superscript “\*” and “a” are significantly different when compared with the group O-I and L-I, respectively at  $p < 0.05$  by One way ANOVA. L-lean, O-obese; L/O-I, O-II, O-III and O-IV are groups that received 2.6, 26, 52 or 129 mg of vitamin A / kg diet as retinyl palmitate respectively

6. Interestingly, up-regulation of *Gfap* in obese rat retina was significantly decreased by vitamin A supplementation with all the doses which is beneficial for retina in treating retinal pathologies. However, vitamin A supplementation had no significant effect on increased expression of *Vegf* due to obesity (Fig 46).

**Fig 46. Expression of *Vegf* and *Gfap* in the retina of WNIN/Ob rats.**

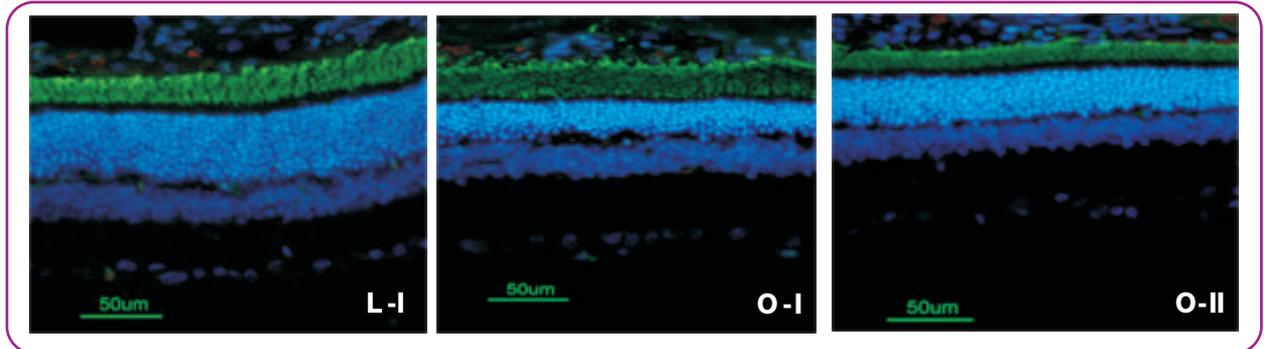
Expression values are determined by qRT-PCR and presented on an arbitrary scale after normalization with *Hgprt*. Data represent mean  $\pm$  SE (n=6). Bars with superscript “\*” and “a” are significantly different when compared with the group O-I and L-I, respectively at  $p \leq 0.05$  by One way ANOVA. L-lean, O-obese; L/O-I, O-II, O-III and O-IV are groups that received 2.6, 26, 52 or 129 mg of vitamin A / kg diet as retinyl palmitate respectively.



7. As reported previously, rhodopsin staining was significantly reduced in WNIN/Ob rats compared with their lean controls. Interestingly the intensity of staining of rhodopsin has increased significantly in O-II group (26 mg/kg diet) in the photoreceptor layer when compared with the O-I group (Fig 47). In conformity with gene expression data, the results with higher doses of vitamin A (O-III and O-IV) were comparable to O-II group.

**Fig 47. Immunohistochemical evaluation of rhodopsin in WNIN/Ob obese rat retina**

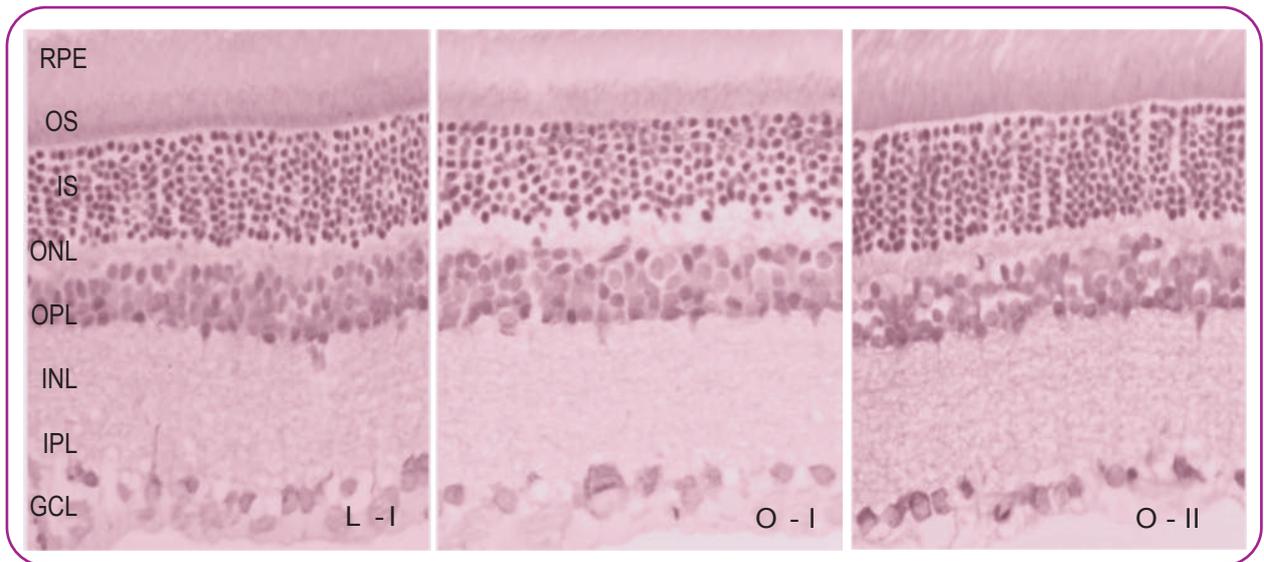
Retinal sections of lean (L-I; 2.6 mg); obese rats that received 2.6 (O-I) or 26 mg (O-II) of vitamin A / kg diet as retinyl palmitate. Nuclei are labeled with DAPI. Scale bar, 50 μm



8. The retina of WNIN/Ob rats had significant alterations such as thinning of outer segment and disintegration of outer nuclear layer compared to lean control. While the overall retinal morphology of lean rats fed with different vitamin A dosages was almost similar, the thickness of outer segment and the photoreceptor layer was comparatively healthy in O-II (26 mg/ kg vitamin A) group when compared with the untreated obese group (O-I) (Fig 48). However, feeding 52 or 129 mg vitamin A did not improve the morphology of WNIN/Ob rat retina further.

**Fig 48. Retinal morphology of WNIN/Ob obese rat retina**

Retinal sections of lean (L-I; 2.6 mg) and obese rats that received 2.6 (O-I) or 26 mg (O-II) of vitamin A / kg diet as retinyl palmitate. RPE-retinal pigment epithelium. OS-outer segment, IS-inner segment, ONL-outer nuclear layer, OPL-outer plexiform layer, INL-inner nuclear layer, IPL-inner plexiform layer, GCL-ganglion cell layer.



9. The alarming increase in the prevalence of obesity all over the world further exacerbates concern about retinal degeneration. Therefore, identifying a suitable animal model and investigating various intervention strategies will aid in combating these progressive blinding diseases. In this regard, WNIN/Ob rat develops increased stress in retinal tissue and progressive retinal degeneration after the onset of severe obesity was reported earlier. Furthermore, vitamin A supplementation resulted in reduced obesity in WNIN/Ob rat. Thus, this novel rat model may be a valuable tool for investigating the effect of vitamin A supplementation on obesity associated retinal degeneration.
10. In this study, it is clear that supplementation of 26-52 mg/kg diet vitamin A alleviated much of the obesity-associated retinal changes in WNIN/Ob rat model.

### CONCLUSIONS

The data indicate that supplementation of 26-52 mg/kg diet vitamin A alleviated much of the obesity-associated retinal changes in WNIN/Ob rat model which may have implications for treatment of retinal degeneration associated with obesity by various dietary factors such as vitamin A.

## VI. FOOD CHEMISTRY

### 1 Nutritional profiling of high yielding popular rice varieties and hybrids of different ecological zones

Rice is rich in genetic diversity with thousands of varieties cultivated in more than 100 countries around the world. It is estimated that there are 1,20,000 different cultivars ranging from traditional rice varieties to the commercially bred elite cultivars. In its natural state, rice comes in many different colours prized for their nutrient and health properties. Rice is tied to cultures and livelihoods symbolizing life and prosperity for billions of people playing a fundamental role in the world food security and socio economic development. To emphasize the importance of rice the UN General Assembly declared 2004 the International Year of Rice under the slogan "Rice is Life".

The biggest public health challenge occurring both globally and in rice consuming countries like India are micronutrient deficiencies of Iron, Zinc, Vitamin A and Iodine which affects more than 3 billion people worldwide. The proportion of global population suffering from micronutrient deficiencies has increased over the last four decades largely due to the increase in acreage under rice and wheat cultivation at the expense of pulse crops, a much richer source of micronutrient and changing dietary habits. India has the highest incidence of undernutrition in the world and among micronutrient deficiencies; iron deficiency anaemia is the most serious public health problem in the country.

In the past, generic food composition data were considered sufficient for most purposes but today the usefulness of cultivar-specific composition data is becoming increasingly acknowledged for understanding diet-related morbidity and mortality. Significant cultivar specific differences have been observed in the nutritional content of rice. Many factors like climate, geography and geochemistry, agricultural practices, post-harvest conditions and handling as well as genetic composition of the cultivar are known to affect the nutrient composition of rice. Among these factors cultivar-specific differences have received the least attention.

India is the largest rice growing country accounting for one-third of the world acreage under rice cultivation. Rice is grown in almost all the Indian states covering more than thirty per cent of the total cultivated area in the country. Rice research in India has traditionally focussed on ways of increasing yield to match the country's burgeoning population and trade. The importance of enhancement of nutritional quality for improving human health through rice breeding is only now coming to the forefront. The International Rice commission (2002) recommended that existing biodiversity of rice varieties and their nutritional composition needs to be explored and that the nutrient content must be among the main criteria used for selection of rice cultivars for use in areas of food insecurity. Rice is an important source of nutrients and breeding rice crops with particularly enhanced nutrient concentration requires knowledge of the variation in the trait among the available germplasm. Therefore this study was initiated in order to document the nutrient composition of 269 high yielding rice cultivars cultivated in India. Nutrient analysis was carried out using brown rice, the raw material for white rice.

#### *Sample processing*

All varieties (*indica* subspecies) of rice were supplied by the Directorate of Rice Research (ICAR), Rajendranagar, Hyderabad in the form of brown rice. Samples were powdered using cyclone mill (UDY Corporation, USA) and stored in clean polyethylene bottles from where aliquots were taken for analysis.

**RESULTS*****Proximate composition***

The macro nutrient composition of all the rice varieties is listed in table 31. All samples had moisture content varying between 6.15 to 11.91 g/100g within the limit of 12 g/100g. Brown rice protein content in 269 cultivars studied ranged from 6.92 to 12.98 g/100g with a mean of 9.43 g/100g. Frequency distribution showed that 45.2% of the samples had protein content below 9 g/100g while only 3% of the samples were above 12 g/100g. The highest protein content of 12.98 g/100g was observed in Phoudum, a traditional high yielding variety. Majority of the samples (51%) had protein content between 9 - 12 g/100g. Brown rice crude fat content in 269 Indian rice cultivars ranged from 1.23 to 3.77 g/100g with a mean of  $2.38 \pm 0.46$  g/100g. The highest fat content of 3.77g/100g found in Kavya variety is substantially. Frequency distribution of brown rice fat content showed that the 73% of the samples had fat content between 2 - 3 g/100g while as many as 30 cultivars had more than 3 g/100g. Though rice is not a rich source of fat the study revealed that considerable variations exist within rice cultivars with some cultivars having substantially higher content that can be utilised to increase fat intake marginally.

The total dietary fiber content analyzed in 105 rice varieties ranged between 3.99 to 4.71 g/100g, with MLT-ME6 having the highest content. The average insoluble fiber and soluble fiber content was  $3.62 \pm 0.16$  g/100g and  $0.79 \pm 0.06$  g/100g respectively. In all varieties, the content of insoluble dietary fiber was significantly ( $p < 0.001$ ) greater than that of soluble dietary fiber. Brown rice ash content in 269 rice cultivars ranged from 0.9 g/100g in Pantdh to 1.99 g/100g in IR36 variety. Mean brown rice ash content was 1.38 g/100g. Frequency distribution showed that 64% of the samples had ash content between 0.9 - 1.5 g/100g while 36% had ash content between 1.5 - 2.0 g/100g reflecting mineral abundance in many rice varieties.

***Mineral Content***

The concentration of elements in 269 brown rice genotypes is shown in Table 1. The sum of nutritionally important minerals assayed in this study represents 36% of the total ash content. In general, mean content of 269 rice cultivars from India had high concentrations of macro element such as phosphorus ( $330 \pm 81$ mg/100g), potassium ( $253 \pm 27.81$ mg/100g) magnesium ( $129 \pm 16$ mg/100g) and calcium ( $13.12 \pm 2.66$ mg/100g). Phosphorus content was highest in T.Basmati (465 mg/100g) and lowest in Aathira (195 mg/100g). Magnesium content ranged from 86 mg/100g in MLT-E-2 to 149 mg/100g in Chageli variety. The variation in calcium content was from 6.8 mg/100g in Aanashwara to 17.11 mg/100g in Pantdhan-12. Mean  $\pm$  SD content of Manganese and copper was  $1.56 \pm 0.35$  and  $0.34 \pm 0.11$  mg/100g respectively. Copper content was low ranging from 0.14 - 0.84 mg/100g. Manganese content was lowest in Aathira (0.77 mg/100g) and highest in MLT-M-11 (2.13 mg/100g). The order of the concentrations of elements in brown rice in this study was phosphorus > magnesium > calcium > zinc > manganese > iron > copper.

Grain iron content ranged from 0.57mg/100g in Aathira to 4.04 mg/100g in MLTE-5 with an average of  $1.36 \pm 0.59$  mg/100g on dry weight basis. Frequency distribution of iron content showed that 46% of the samples had less than 1mg/100g, 48% had 1 - 2 mg/100g and 6% had more than 2mg/100g. The coefficient of variation observed for grain iron content in the present study was as high as 43% which indicates ample room for improving rice iron content.

Grain zinc content ranged from 1.46 mg/100g in Lalat to 3.87 mg/100g in MLT-M-14 with a coefficient of variation of 19%. Frequency distribution showed that 73% of the samples had zinc content in the range of 1 - 2 mg/100g and 14% had more than 2 mg/100g. The width between the lowest and highest zinc content in the present study was 2.41 mg/100g while that of iron was much higher at 3.47mg/100g. Rice is not a rich source of iron and zinc but it remains the major source of intake for these micronutrients in the rice eating Indian population.

**Table 31. Proximate composition, dietary fiber and mineral content in 269 high yielding Indian rice cultivars**

Parameter	n	Mean $\pm$ SD	Range
Moisture g/100g	269	9.69 $\pm$ 1.37	6.15 - 12.66
Protein g/100g	269	9.47 $\pm$ 1.22	6.92 - 12.98
Fat g/100g	269	2.36 $\pm$ 0.46	1.23 - 3.77
Ash g/100g	269	1.39 $\pm$ 0.18	0.90 - 1.99
Insoluble dietary fibre g/100g	205	3.62 $\pm$ 3.64	3. 13.90
Soluble dietary fibre g/100g	105	0.79 $\pm$ 0.06	0.66 - 0.92
Total dietary fibre g/100g	105	4.41 $\pm$ 0.17	3.99 - 4.71
Energy g/100g	105	347 $\pm$ 3.64	340 - 356
Carbohydrate g/100g	105	71.79 $\pm$ 1.37	68.04 - 75.77
Energy Kcal	105	347 $\pm$ 3.64	340 - 356
Iron mg/100g	236	1.23 $\pm$ 0.53	0.52 - 3.75
Zinc mg/100g	236	2.38 $\pm$ 0.45	1.01 - 3.46
Copper mg/100g	236	0.31 $\pm$ 0.10	0.13 - 0.78
Manganese mg/100g	236	1.41 $\pm$ 0.31	0.75 - 2.46
Calcium mg/100g	236	12.27 $\pm$ 2.59	8 - 19
Magnesium mg/100g	236	116 $\pm$ 14.04	69 - 150
Phosphorus mg/100g	236	297 $\pm$ 71.18	113 - 4987
Calcium mg/100g	104	253 $\pm$ 27.81	162 - 347

### *Amino acid content*

Box plot showing distribution of various amino acids in 42 high yielding rice cultivars is shown in Fig 49. The total essential amino acids made up 39% of the total amino acids. Lysine content ranged from 3.42g/100g in AP 41 Kanchana to 4.2g/100g in AP 71 Pratap with a mean of 3.76  $\pm$  0.20 g/100g protein. Besides AP71 Pratap another two varieties AP 85 Sarala and AP106 also had lysine content above 4 g/100g protein. The mean  $\pm$  SD of threonine content, the second limiting amino acid in rice was 3.45  $\pm$  0.21 g/100g protein. The lowest threonine content of 3.03 g/100g protein was observed in IR 64 while the highest content of 3.86 g/100g protein was found in AP 85 Sarala. After lysine and threonine, isoleucine is the only amino acid that can sometimes be limiting in rice protein. Isoleucine content ranged from 3.02 - 4.59 g/100g protein with mean content of 3.91  $\pm$  0.39 g/100g protein. The savoury amino acids glutamate and aspartate was the major amino acids constituting 19.36  $\pm$  0.39 and 8.91  $\pm$  0.38 g/100g protein respectively. The sweet amino acids glycine and alanine was as high as 4.51 $\pm$ 0.20 and 6.01 $\pm$ 0.29 g/100g protein respectively. The range of the different essential amino acids expressed in g/100g protein was 1.32 - 1.54 for tryptophan, 1.51 - 3.04 for cysteine, 4.05 - 6.92 for valine, 1.11 - 2.40 for methionine, 7.23 - 8.73 for leucine, 5.12 - 5.75 for phenylalanine, 2.23 - 2.73 for histidine and 7.53 - 8.62 for arginine. There was reasonable level of variation for all the amino acids indicating that genetic gain by means of selection is likely.

### *Fatty acids composition*

Box plot showing the distribution of various fatty acids in 85 Indian rice cultivars is presented in Fig 50. The major fatty acids were Palmitic (range 20 - 26), Oleic (range 30 - 37) and Linoleic acids (33 - 42) which accounted for more than 92% of the total fatty acids. The Mean  $\pm$  SD content of Myristic, Stearic and  $\alpha$ -linolenic acids was 0.32  $\pm$  0.06, 2.63  $\pm$  0.50 and 1.52  $\pm$  0.23 respectively.  $\alpha$ -linolenic content ranged from low of 0.93 to as high as 2.19%. Capric acid was detected at very low levels ranging between 0.02 - 0.32 % in the present study. Low levels of Arachidic (0.45  $\pm$  0.07), Behenic (0.19  $\pm$  0.06), Lignoceric (0.27  $\pm$  0.12), Nervonic (0.07  $\pm$  0.02) and

Fig 49. Box plot showing the distribution of various amino acids in 42 high Yielding Indian rice varieties

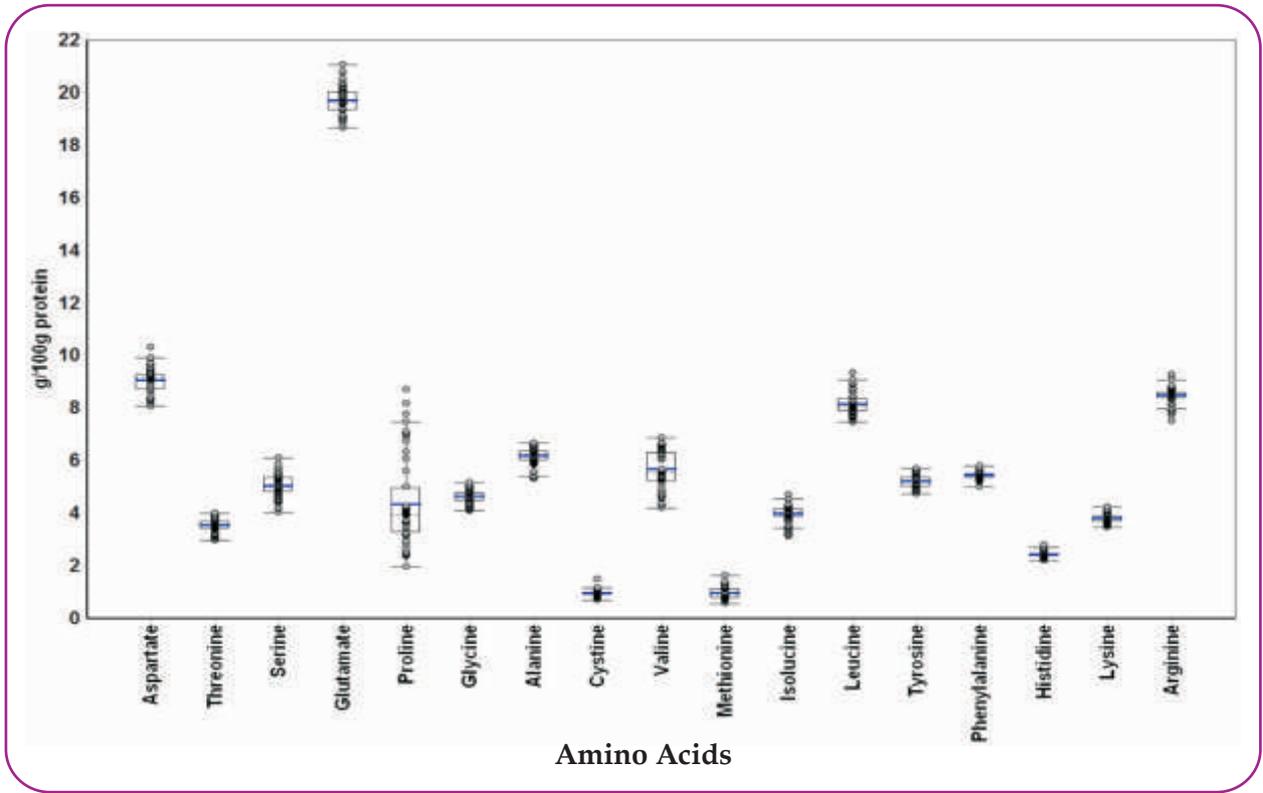
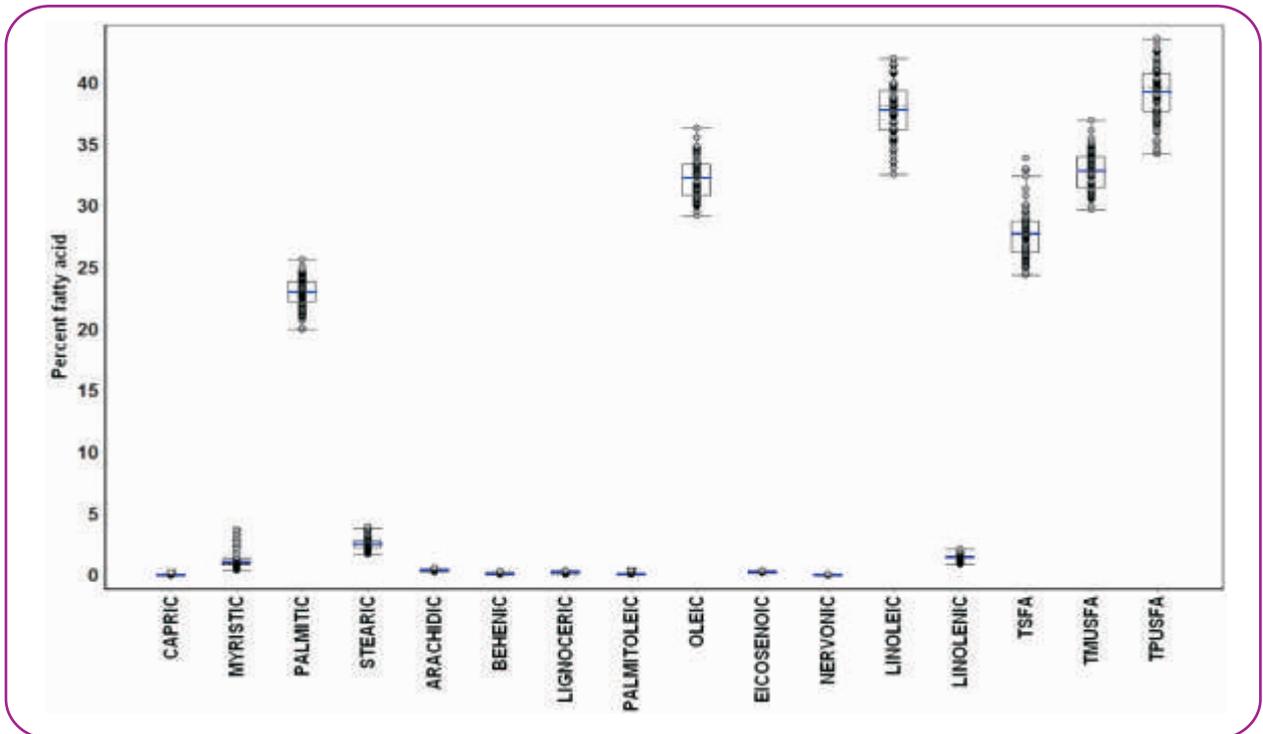


Fig 50. Box plot showing the distribution of various fatty acids in 85 high yielding Indian rice cultivars



Eicosenoic ( $0.32 \pm 0.06$ ) acids were also observed in all the rice samples studied here. The wide range of individual fatty acids in the diverse rice varieties shows that rice breeding can be designed to increase individual fatty acid of interest for improved health benefits.

## **CONCLUSIONS**

The study has revealed that the existence of diverse rice varieties with wide range of nutrients within rice cultivars in India. The importance of nutrient diversity in rice is being understood now with the realization that biodiversity is fundamental to food and nutrient security of the people. The various agro climatic regions in India offers immense opportunity for proper selection of rice cultivars for certain regions which combine high mineral levels with low levels of inhibitors for improved bioavailability in order to combat micronutrient deficiencies in the country.

## VII. EXTENSION AND TRAINING

### A. SERVICE ACTIVITIES

#### 1 PUBLICATIONS

The popular publication “Dietary Guidelines for Indians – A Manual” which was under revision for quite some period was revised and reprinted. The updated version of “Diet and Diabetes” and another publication “NIN 1918-2011: Nine decades of leadership in nutrition research” are in press for printing. The other publications which were reprinted, on popular demand include Nutritive Value of Indian Foods, Some Therapeutic diets, Dietary Guidelines for Women and Children (Telugu), Low Cost Nutritious Supplements, Nutrition for Mother and Child, Low Cost Balanced Diets (North and South) and Fruits.

#### 2 TRAINING PROGRAMMES

##### *Regular Training Programmes*

A total of thirty eight candidates have attended the regular training programmes of the Institute viz. (i) MSc (Applied Nutrition) III Batch 2011-12 – 15 participants (ii) Post-Graduate Certificate Course in Nutrition - 13 participants (iii) Annual Training Course on Endocrinological Techniques and their Applications - 10 participants.

The Mini-Convocation for the 1<sup>st</sup> Batch of MSc (AN) was held on Feb. 2, 2012, certificates were awarded to the successful candidates and also Dr.BK.Nandi fellowships and prize was given to the meritorious students.

#### 3 EXTENSION ACTIVITIES

##### *3.1 Exhibitions*

- ❖ Organised an exhibition on Nutrition and Health in Bharat Utsav as part of Public Awareness Campaign to create awareness about the advancement of science at the community level, in an event organized by Rural Action, Integrity and Social Action (RAISE), I & PR Dept, Govt. of India (Aug. 18-24, 2011).
- ❖ Participated in 99<sup>th</sup> Indian Science Congress, held at Bhuvaneswar and organized a exhibition stall, at ICMR pavilion. Explained the posters on nutrition and health to various groups of visitors to the pavilion (Jan. 3-7, 2012).
- ❖ Organised an exhibition of Nutrition and Health and also publication sales counter at People's Plaza, Necklace road as part of the Millet Food festival, organized by ANGRAU in association with Commissionerate of Agriculture, Hyderabad (March 24-26, 2012).

##### *3.2 Popular Lectures/Awareness Camps*

- ❖ Delivered an extension lecture on “Nutrition and Health” to the Police personnel of different cadres, Inspectors and DSP's of CM security wing at Moinabad, RR Dist., organized by Intelligence Wing of AP Police Department. About 60 police personnel participated in the programme (June 7, 2011).
- ❖ Delivered an extension lecture on “Nutrition and Health” to the members of the Open Home, organized by LIFE NGO, Hyderabad. About 40 members participated in the programme (June 15).



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- ❖ Delivered a lecture on “Nutrition and Health” for the retired teachers of Andhra Pradesh Agricultural University (APAU), Rajendranagar, Hyderabad. About 100 retired teachers/professors from AP Agricultural University and Veterinary College participated in the programme (June 19).
- ❖ Delivered lecture to the Police Officers of CISF on “Food, Nutrition, Health”, at CISF Head Qrts, Shameerpet, Hyderabad. About 40 inspectors participated in the programme (June 20).
- ❖ Delivered lecture on “Nutrition and Health” for Intelligence security wing of CM security, Integrated Intelligence Training Academy, Moinabad (Aug. 02).
- ❖ Delivered lecture on “Nutrition and Health” for Intelligence security wing of CM security, Integrated Intelligence Training Academy, Moinabad (Oct. 13).
- ❖ Delivered a lecture on “Nutrition, Food Habits and Weight Management” to the ISW and District Police personnel of IX Batch Basic Foundation Course, at Integrated Intelligence Training Academy, Moinabad, Hyderabad (Nov. 22).
- ❖ Delivered a lecture on “Food habits and weight management” to the Police Personnel participants of 10<sup>th</sup> Batch Basic Foundation Course for ISW, at Integrated Intelligence Training Academy, Moinabad, Hyderabad (Jan. 19, 2012).
- ❖ Addressed village people and students of Andhra Mahila Sabha, Osmania University on Nutrition and Health during the special NSS winter camp at Parvathapuram, Uppal, Hyderabad. About 100 students and 50 villagers attended the programme (Jan. 22).
- ❖ Addressed parents of VIP international school on various aspects of Nutrition and Health, Saidabad. About 125 parents participated in the programme (Feb. 25).
- ❖ Participated in a Science Education Fair, at Narayana Concept School, Shah Ali Banda, Hyd and delivered a lecture on the importance of science in day to day life. About 300 school children and 20 faculty members participated in the programme (March 10).

### 3.3 Radio Talks and TV Programmes

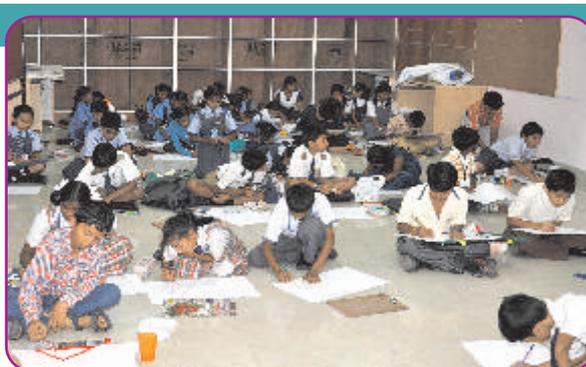
- ❖ Delivered a lecture on the Importance of National Nutrition Week celebrations and the activities organized by NIN during this occasion as a radio talk on September 1, 2011.
- ❖ Delivered a radio talk on Balanced Diet and Importance of Fat for Industrial workers on September 5, 2011.
- ❖ Role of Nutrition in Health Part 1 and Part 2 (Sept. 6 & 7, 2012).
- ❖ Participated as a subject expert in the discussion organized by Doordarshan, Ramanthapur, Hyderabad as part of National Science Day Celebrations (Feb. 9, 2012).
- ❖ Explained about the MSc (AN) programme and it’s prospects for the students opting this programme in career plus programme organized by TV9 (March 28, 2012).

## 4 SPECIAL EVENTS

### 4.1 National Nutrition Week Celebrations (1-7, Sept. 2011)

In connection with the National Nutrition Week celebrations, the following programmes were organized:

- ❖ A Painting Competition was held for the school students of class I - X on Nutrition related themes. Over 170 students from



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different schools participated in the contest under Sub-junior (I-IV classes), Junior (V-VII classes) and Senior (VIII-X classes) categories (Sept. 2).

- ❖ A symposium on “Nutrition Education for New Generation – Opportunities and Challenges” was organized in collaboration with Food and Nutrition Board, Government of India. The technical session of the symposium had academics and researchers speaking on various topics like Positioning of Nutrition and Health messages in Media, Adolescent Nutrition and Nutrition Communication (Sept. 2).
- ❖ A Symposium on “Nutrition Security for All – An Emerging National Imperative” was organized by NIN. The technical session witnessed talks by experts on revised dietary guidelines for Indians; right to food act; food processing and nutrition security (Sept. 6).
- ❖ A school-based nutrition education program was organized at the Richmond's High School, Srinagar Colony, Hyderabad on 7<sup>th</sup> Sept. 2011. The students of MSc 2<sup>nd</sup> year, NIN enacted a short skit to educate the high schoolers on right nutrition and healthy lifestyles. This followed by a lecture-cum-interactive session. About 200 students participated (Sept. 7).
- ❖ A Nutrition Awareness Programme was organized by the scientist of Extension and Training Division in association with Railway Department at Railway Institute, Moula Ali, Hyderabad. A nutritious recipe contest for women and poster contest for school children were also organized as part of the programme, which culminated with the nutrition quiz competition for schoolchildren. About 300 women, children and employees took part in the programme (Sept. 7).
- ❖ A nutrition education programme was organized for the benefit of adolescent school girls in Railway High School, Mettuguda. About 200 girls and their science teachers participated in the event (Sept. 8).

### 4.2 *World Food Day (Oct. 16, 2011)*

As part of the World Food Day celebrations, a one day symposium was organized on “Food prices – From crisis to stability”, by Association of Food Scientists and Technologists, Hyderabad Chapter and Oil Technologists Association of India.

### 4.3 *National Science Day Celebrations (Feb. 28, 2012)*

In connection with the National Science Day celebrations, a lecture by Prof. Asim Duttaroy, University of Oslo, Norway on “Dietary fatty acids and foeto-placental growth and development” was organised.

### 4.4 *International Women's Day (March 8, 2012)*

A lecture was delivered on the “Importance of nutrition for adolescent girls, pregnant and lactating women” at the celebrations organized by Sanghi College. 50 women from different walks of life participated in the programme. Also about 70 college girls participated in the programme.



Prof. Asim Duttaroy, University of Oslo, Norway delivering a lecture

**B. RESEARCH ACTIVITIES**

1

**Assessment of intra and extra individual factors on food consumption pattern in rural population – a diagnostic model approach**

Consumption of nutritious food is a major factor to ensure good health, which is being influenced by multiple factors such as income, food preference, beliefs, practice, culture, environment, food availability, marketing facility, literacy etc. The FAO food balance sheet indicates that the per capita calorie availability has increased in developing countries like India. At the same time, micronutrient deficiency disorders have become a public health problem in our country. It is also projected that, by 2020, chronic diseases will account for almost three-quarter of all deaths worldwide and that 71% of deaths would be due to ischaemic heart disease (IHD), 75% of deaths due to stroke and 70% of deaths due to diabetes will occur in developing countries (*WHO TRS Report 2003*). Hence, prevention of these diseases by bringing awareness and attitudinal change in population is economical than the curative approach.

Over half of the children under the age of five years in India are moderate or severely malnourished, 30% of newborn are born underweight and nearly 60-70% pregnant women are anaemic. This situation prevails despite the country having attained self-sufficiency in food production for well over a decade. At the same time, due to major alterations in lifestyles and dietary intake, there is a consequent increase in prevalence of obesity and non-communicable diseases. In this scenario, focus will be on nutrition education and high priority is to be accorded to monitor the ongoing demographic development, economic transition and etiological and lifestyle changes – nutrition and health status of the population.

Hence, general awareness about health and specific knowledge about nutrition are very much essential for women especially because they are managing the family including cooking and distribution of food to the family members. The knowledge about nutrition would enhance good cooking practices so that they can provide better nutritious food for their family. Keeping this in view, this study was proposed with the following objectives:

**OBJECTIVES***Phase 1*

- ❖ To identify the factors affecting food habits and food intake in the village population.
- ❖ To find out the attitude, nutrition awareness and behaviour about food consumption among the villagers.
- ❖ To develop appropriate nutrition education in the local language (Regional language).

*Phase 2*

- ❖ To devise training methods to train the PG students on nutrition after assessing their knowledge who in turn would be educating women in the villages.
- ❖ To measure the attitudinal changes among the village women before and after the intervention.

**MATERIALS AND METHODS***Research design*

It is a descriptive research design to find out various determinant factors in food consumption. By adopting purposive sampling method the women studies department faculty

members as well as students of Department of Women Studies were selected from Alagappa University from Tamil Nadu for the study.

Training was given to the students of women studies on community nutrition research. First year PG students were recruited for this research and they continued the research work for one and half years from starting to end of this research study, and this work was considered as a project work for their academic purpose. Six villages with 500-1000 population were selected at random near the University. The women of 18-45 years including pregnant and lactating women were recruited for the study.

## RESULTS

The socio-economic and religious aspects of the respondent revealed that majority belong to Hindu religion. Next to Hindu religion Muslims are 31.3 in percentage. There were very few from other religions. Approximately 62 % of the respondents were in under <₹ 20,000 income per annum group. A majority of them were agricultural laborers and industrial workers. About 26 % of the people were in ₹ 20,000-30,000 income group. It is also noted that majority of the respondents were from Backward and Scheduled Caste communities. Safe drinking water and electricity connection was also well maintained in these villages. Hardly 50% of the respondents had the sanitary facilities; the remaining used the open toilet system. Firewood and biogas were the major cooking fuels used by them.

The knowledge about anemia, symptoms of anemia, impact of anemia during adolescent age and pregnancy was very minimum. Almost 80 % of them didn't know about anemia though they were taking leafy vegetables twice a week and flesh foods once a week. (Table 32)

### Infant Feeding

Interviews were conducted with mothers of children below 5 years. About 22% perceived their children as healthy. When interviewed about the disease prevalence, under the age of five, it was observed that cold, fever and diarrhea was more prevalent. When asked about the causes of these disease, the respondents said that it was impure water, poor hygienic practices and infection. Very few women knew that it could also be due to inadequate food. About 17 % of the mothers said that exclusive breast feeding should be up to six

**Table 32. Effect of intervention on food practice**

No	Name	Mcneemar test value	Significant/not significant
<b>CEREALS</b>			
1	Wheat	0.000	S 0.01
2	Jowar	0.000	S 0.01
<b>PULSES AND LEGUMES</b>			
3	Bengal gram	0.001	S 0.001
4	Channa	0.000	S 0.001
5	Beans	0.000	S 0.001
6	Groundnut	0.000	S 0.001
<b>NON VEG</b>			
7	Chicken	0.000	S 0.001
8	Egg	0.00	S 0.001
9	Mutton	0.000	S 0.001
<b>MILK AND MILK PRODUCTS</b>			
10	Curd	0.000	S 0.001
<b>FRUITS</b>			
11	Mango	0.000	S 0.001
12	Papaya fruit	0.000	S 0.001
13	Orange	0.000	S 0.001
14	Guava	0.000	S 0.001
<b>VEGETABLES</b>			
15	Pumpkin	0.000	S 0.001
16	Glv	0.000	S 0.001
17	Ridge guard	0.000	S 0.001
18	Snake guard	0.42	S 0.05
19	Cluster beans	0.000	S 0.001
20	Bottle guard	0.000	S 0.001
21	Lemon	0.000	S 0.001
<b>OILS</b>			
22	Gingely oil	0.000	S 0.001
23	Palm oil	0.000	S 0.001
24	Ghee	0.000	S 0.001

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months. As per their cultural practices they were initiating supplementary food within six to eight months. About 17% of the mothers reported to have used infant formula food. Nearly 6.5% women were giving ragi porridge as a supplementary food.

### *Educational Material developed for Intervention*

Based on the baseline data collection, nutritional knowledge lacunae was identified. Accordingly, nutritional educational material like folder, charts, flip charts and flex charts were developed for the first intervention and for the second intervention, a 32 minute CD was developed by using these material and two time points education was given to the respondent of the selected villages of the experimental group. For three months period every week the staff of Alagappa University, Department of women studies, gave nutrition education to the experimental villages and as the principal investigator elaborated lectures were given. To carry out the second intervention a 32 minutes nutrition information CD was screened for the experimental villages. In addition to the film, information lecture was also given and adequate lecture was given to them to clarify their doubts as a second intervention. After this, the practice measurement schedule was administered to find out the changes in the food practices. Results are given here.

According to the McNemars test, the above tables clearly reveals the intervention's efficacy. But in some areas even though the same methods were used, there was no significant improvement observed during the baseline survey. It was observed that the main staple food was rice. Geographically, the research area is near to the seashore area namely Pudukottai (40 km) and Ramanathapuram (52Km) and most of the respondents are from Backward and Scheduled caste communities and they consume fish as the main food. Hence, these food items are taken regularly which is why there was no improvement in these food practices. The results have indicated that they have added many food items in their diet. Even though they have altered their food habits they need knowledge about Anemia and its health effect on women. Therefore, more information should be given about Anemia and its prevention (Table 33).

The results indicated that all the income groups consume rice as major staple food followed by wheat (45%). Whereas, it was observed that 34.5%, 78.5% and 75.7% were not consuming ragi, jowar, bajra respectively in their diet. Regarding the consumption of pulses, 45% were consuming 'tur' dal in their daily diet. Other pulses like green gram, black gram and channa were consumed twice in a week (45%). Daily milk intake in all income group is as high as 94.167 (average) but it was consumed in the form of tea and coffee.

**Table 33 . No improvement in food practice even after intervention**

No	Name	Mcneamar test value	Significant/ Not significant
<b>CEREALS</b>			
1	Rice	-	NS
2	Ragi	-	NS
3	Bajra	0	NS
<b>PULSES AND LEGUMES</b>			
4	Pulses and nuts	0	NS
5	cashew nut	-	NS
<b>NON VEG</b>			
6	Fish	0	NS
<b>MILK AND MILK PRODUCTS</b>			
7	Milk	0	NS
8	Buttermilk	0	NS
<b>FRUITS</b>			
9	Banana	0	NS
10	Apple	0	NS
<b>VEGETABLES</b>			
11	Carrot	0	NS
12	French beans	0	NS
13	Brinjal	0	NS
14	Ladys finger	0	NS
<b>OILS</b>			
15	Sunflower oil	0	NS
16	Vanaspathi	0	NS

## Educational Intervention Material

Nearly 16 percent of the women were illiterate, 10.6 percent were literate till primary, 54.2 percent were literate up to secondary level. Only 7.7 percent were undergraduate and above level. Extreme income variation was observed among the villages. 89 Percent of them were earning less than Rs. 40000/- per year.

Government infrastructure like health care facilities, road infrastructure, educational institutions, integrated child development centers, medical shop availability and marketing facilities are directly linked with social development. In this study approximately 98 % of the villages have ICDS facilities. 73 % of the villagers have Primary Health Care Centers and in control villages 75% are found with health care facilities. Similarly to meet out the emergency health care needs the villages have private health care centers also. Within 1-3 km radius, medical shops are also available. About 95% are using the Government Ration shop for groceries like cereals, sugar, oil, kerosene etc. Now regarding the marketing facilities, weekly vegetable markets are made available commonly known as *Rythu Bazaar*. All the villages are well connected with the nearby towns and villages. Regarding the educational facilities, about 36.5 % of village have primary school and Government High schools. Colleges and universities are also available within 10 km radius distance.

More expenditure was on cereals (81.4) followed by milk and milk products (63.46) followed by Non-veg (51.87) food items. At the same time the expenditure on processed food (38.16), fruits (46.36), and oil (48.23) were moderate. The amount spent on Non-veg (51.87) item was greater than that spent on fruits (46.36) and vegetables (39.1). The analysis indicates there was no significant difference in the expenditure pattern among the village population. It was observed that there are no significant differences in control experiment villages. The post intervention data entry was completed. Analysis of the same is in progress.

## CONCLUSIONS

The continuous and repeated different methods of communication with local language inter personal communication and group discussion has brought about a modification in their food practice. To continue the sustainability of the program constant collaboration with women study center will be ideal to create awareness about health and nutrition.

2

## Influence of mass media advertisements on family food purchasing patterns and efficacy of behavior change intervention

Mass media plays a pivotal role in information, education and communication. Each kind of media has got its own role to make an impact on the human mind. The number of newspapers and magazines in the market and the number of channels on television has increased tremendously. Commercials are telecasted between popular programmes thereby reaching a wide audience. They also appear in newspapers and will get the readers attention by the sheer size of adverts or by catchy captions or by positioning.

Children usually fall prey to advertisements of processed foods which entice them with free gifts. These foods can lead to many health problems. TV advertisements use subjective camera angles; clever editing and child artists to make advertisements appealing and thereby

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influence viewers to buy these products. Advertising uses appeals as a way of persuading people to buy certain products. Appeals are designed in a way so as to create a positive image of the individuals who use certain products. Advertising agencies and companies use different types of advertising appeals to influence the purchasing decisions of people.

The most important types of advertising appeals include emotional and rational appeals. Emotional appeals are often effective for the youth while rational appeals work well for products directed towards the older generation. Using data from Nielsen Media Research, two recent unpublished studies have also attempted to document the number of food ads that are actually seen by children (Ippolito, 2005; Collier Shannon Scott, 2004). However they do not offer information on the *content* of food advertising, such as whether or not the ads portray physical activity, the nature of the appeals used, the target audience, references to health claims or use of premiums, contests and sweepstakes.

Content analysis of advertisements related to food items including oils, processed foods, health drinks and other beverages and studying their impact on vulnerable groups such as children and their mothers has not been done in the Indian context. Based on the content analyses, nutrition education will be planned to guide the mothers make right food choices. Since, women are usually more involved in making food purchases for the family and also in home management, getting their perceptions on various food related advertisements and educating them will benefit the entire family. Keeping this in view a nutrition education intervention study was proposed with the following objectives;

### OBJECTIVES

- To assess time spent by women and children (8-14 yrs) in urban (middle class) population on mass media.
- Compile adverts related to food and beverages based on viewership/ readership and do content analyses.
- To assess the influence of advertisements on women's purchasing behaviours
- To assess the food consumption patterns (main meals and snacking) of children and mothers.
- Develop appropriate communication material to educate the selected population.
- Provide intervention twice over a six month period and evaluate the efficacy of the intervention package in changing purchase behaviours.

### MATERIALS AND METHODS

Three study groups of adequate sample based on the pilot study findings was included. Two schools were selected from the state board syllabus and one school from the Central Board syllabus. A food frequency questionnaire and a TV viewing habit questionnaire was developed and interviewed with the school children.

Six middle income urban apartment members were included in the study. Reproductive age group women were interviewed regarding media exposure and food purchasing pattern.

The pre-tested interview schedule was used for baseline data collection. The women's Nutritional knowledge was measured. About 105 women were interviewed.

### Work done

*Educational intervention for school children* – Based on the baseline data information, educational material was developed. One set of folders and a CD “You are what you Eat” containing nutritional information was used to educate the students. In Addition to that, the power

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point containing all topics why we need energy, energy rich food, protein, protein rich food, importance of micronutrient and importance of physical activity was used to educate the children.

After 15 days time the same knowledge assessment questionnaire was administered among the school children to find out the knowledge improvements. The results indicated that match the following and fill in the blanks. They scored less marks, where as in other areas knowledge increment was observed.

Based on the women's baseline data a knowledge assessment questionnaire was developed and pre-tested. Knowledge assessment survey was completed. Nutrition education interventions were carried out with folders and lecture for the selected women those who were included in the study. The intervention lecture covered all the major nutrition topics, as well as food habits and beliefs. Adequate time was given to them to clarify their doubts.

### RESULTS

#### *Socioeconomic status*

The mean age of the students from State Education Board school was  $13.2 \pm .52$  and that of CBSE affiliated school was  $12.9 \pm .61$ . Mean height was  $153.9 \pm 8.2$  and  $155.5 \pm 7.7$  respectively. The mean age and height of the children did not differ significantly. The mean weight of children from State Education Board school was  $44.1 \pm 10.0$  and central board was  $46.5 \pm 11.1$  the difference in the mean weight was significant ( $p < 0.05$ ).

Parental education and occupation was used as proxy for socioeconomic status. Father's occupation was comparable among both the two schools. 43.3% of the children from the State Education Board School had fathers working in Government sector while there were 39% in the CBSE affiliated School group. 39.4 % (State Education Board School) and 48.2% (CBSE) fathers worked in private firms. 37% and 43% of them were professionals (Doctors, Engineers or Lawyers) and 52 % (State Education Board) and 57% were graduates. Nearly 75% of the mothers of children from both schools were home makers and 56% of them were graduates. Parental education and occupation were found to be comparable among these two schools.

About sixty percent of the students from State Education Board school and 50% from CBSE affiliated school were boys.

#### *Nutrition knowledge on different aspects*

There were significant differences between the two schools (Table 34) in knowledge about proteins and fats. Results showed that children following the central board syllabus had significantly higher knowledge regarding sources and functions of protein; they also had significantly higher knowledge regarding foods that have high fat content and foods that contain trans fat compared to children following the state syllabus.

**Table 34. Knowledge about proteins and fats**

Knowledge items Proteins and Fats	State Board School	Central Board School	P value
Body building foods	35.3%(30)	72.4%(89)	0.001
Protein rich foods	56.5%(48)	73.8%(90)	0.007
High Fat foods	43.5%(37)	81.3%(100)	0.000
Foods with trans fats	40.0%(34)	61.8%(76)	0.002

Knowledge about the sources of various micronutrients and their functions was comparable among both schools. However, children from CBSE affiliated school had significantly higher knowledge regarding sources of vitamin B and also higher (trend 0.06) knowledge

**Table 35. Knowledge about micronutrients**

	State Board School	Central Board School	P value
Main sources of micronutrients	95.3% (81)	95.9% (118)	*NS
Sources of Vit A	56.5% (48)	67.5% (83)	NS
Sources of Calcium	89.4% (76)	87.8% (108)	NS
Mineral for bone strength	72.9% (62)	83.7% (103)	NS (.06)
Sources of Vit C	31.8% (27)	44.7% (55)	NS (.06)
Sources of Vit B	30.6% (26)	51.2% (63)	.003
Rich source of iron	72.9% (62)	72.4% (89)	NS

\*NS- Not Significant

Table 36 shows the results of knowledge regarding micronutrient deficiencies related disorders. Children from the CBSE affiliated school have significantly higher knowledge regarding the consequences of various micronutrient deficiencies. Only 4.7% children from the State Education Board knew iron deficiency causes anemia compared to the 42.3% of children from the school affiliated to CBSE.

**Table 36. Knowledge about micronutrient deficiencies**

	State Board School	Central Board School	P value
Iodine Deficiency	50.6% (43)	69.1% (85)	0.007
Vitamin D	43.5% (37)	81.3% (100)	0.000
Vitamin A	60.0% (51)	87.0% (107)	0.000
Vitamin C	35.3% (30)	66.7% (82)	0.000
Iron Deficiency	4.7% (4)	42.3% (52)	0.000

## RESULTS

A total of 1602 food advertisements were appeared during the sample period in all the four television channels viz., Cartoon Network, Disney, STAR plus and Gemini. Total duration of these advertisements was calculated as 42,120 seconds. Advertisements about chocolates and sweet products were telecast maximum no. of times followed by the adverts on Health/ Energy drinks and Grain-based products (Table 37). Advertisement of chocolates/sweets, Biscuits/cookies and potato crisps were mostly telecast in the children's channel compared to mainstream, which clearly indicates that the manufacturers of these products had targeted child audience. Contrary to this, 63% of adverts on Health/ Energy drinks were seen in the mainstream channels, which audience consist mostly adults including house-wives or parents. As the cost of health/ energy drinks are high compared to the chocolates and the decision to purchase these drinks lies only with parents, these products are mostly advertised in the mainstream channel. Almost all the adverts on health/energy drink boosted these drinks as inevitable for the growth of children which tempts the parents. The same was the case with advertisement on grain/food-based food products. 74% of this product advertisement was seen in the mainstream channels. All the advertisements of potato crisp during the sample week was appeared only in the children's channel and all the adverts on tea/coffee was seen only on mainstream channels which can be perceived as symbolical division of food habits between children and adults.

### Media using habits of women

The average income of the respondent was Rs. 25076.9. About 38.5% women had the BMI in the range of 23-27 and about 45.2% were above 27. The remaining people fell in the under

Table 37. Statement showing classification of food advertisements telecasted in various television channels from 23-29 October, 2010

Product	No. of advertisements						Duration of advt. (in seconds)						Health/ Nutritional claims
	Cartoon Network	Disney	Star Plus	Gemini	Total	Cartoon Network	Disney	Star Plus	Gemini	Total			
Biscuits/ Cakes	61	16	24	-	102	1220	430	260	-	1910	Complete health/ high protein		
Chocolate/ sweet products	114	226	122	32	494	2790	6530	3570	680	13570	More nutrients		
Health/ energy drinks	36	48	76	64	224	490	2450	1180	2160	6280	Makes taller & sharper		
Dairy	-	18	138	-	156	-	680	4900	-	5580	Keeps you healthy		
Tea / Coffee	-	-	26	34	60	-	-	500	600	1100	Makes you active		
Grain/ Fruit based food products	58	-	64	98	220	980	-	2170	2560	5710	Calcium; Carbohydrate s		
Atta/ Noodles /Pizza	50	42	68	14	174	770	1220	1470	300	3760	Low calories		
Potato chips	12	14	-	-	26	190	380	-	-	570	-		
Oils/ Nuts	-	12	18	18	48	-	370	600	500	1470	Tasty and healthy		
Others	74	12	-	12	98	1380	380	-	410	2170	-		
<b>Total</b>	<b>406</b>	<b>388</b>	<b>536</b>	<b>272</b>	<b>1602</b>	<b>7820</b>	<b>12440</b>	<b>14650</b>	<b>7210</b>	<b>42120</b>			

## vii extension & training

nourished categories. About 45.2% had studied up to graduation level and about 22.2% were up to post graduation. The remaining were inter and primary. Exactly 50% of the women felt that television advertisements were informative and 48% of the women agreed that the advertisements were influencing their food purchasing patterns, especially it was influencing the purchase of oils, snacks, wheat flour and aerated drinks. Because of the television advertisements, 86% of the families used only sunflower oil as the cooking oil.

### **CONCLUSION**

The results indicated that the time spent on television viewing by women and children influence their family food purchasing pattern including the snacking habits of children. Effective planning and strategy was essential to propagate the theme of eating variety of natural foods and to minimise the intake of processed food.

## VIII. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

### 1 Assessment of consumption of processed and non-processed foods in India

Safe and nutritious food is the basic requirement of human beings. However, food is likely to be contaminated during the growth, harvest, process, transport and distribution with pesticides and other chemicals. Consumption of foods having such contaminants in amounts other than permissible limits, for prolonged periods may pose several health problems. In order to assess the extent of such risks to which the communities are exposed in our country, it is necessary to identify and quantify various chemicals and pesticides present in the processed and non-processed foods and to assess the extent of their consumption by the communities in different regions of the country. Such data is vital in planning and implementing suitable public health programmes to ensure safety and quality of food available for public consumption. The Joint Parliament Committee, therefore, recommended (JPC Report under 2.184) that a database be generated on food consumption pattern of the community in urban as well as rural parts of India.

#### METHODOLOGY

For the purpose of the study, “processed food” was defined as foods that are subjected to technological modifications either for preservation or for conversion into ready-to-use or ready-to-eat-foods, which includes foods such as “ready mixes, dehydrated foods, pasta products, canned foods, confectionaries, bakery products, dairy products, breakfast foods etc” (ICMR 2010).

**Study design:** It was a community based and cross sectional study, covering two major seasons of the year. The survey was carried out by adopting multistage stratified random sampling method. For the purpose of the study, the entire country was divided into 5 geographical regions i.e. North, South, East, West and North East.

**Sample size and Sampling procedures:** Based on the resources available and in order to capture as much of the variation in the community, the following sampling design and size was adopted for rural and urban areas.

**Nutritional Assessment:** All the subjects available in the selected 3000 households (HHs) from rural and 1500 HHs from urban areas at the time of survey were covered for anthropometry to assess nutritional status.

**Measurement of blood pressure:** All the available adult individuals in the selected households were covered for measurement of blood pressure.

**Estimation of Fasting blood glucose:** Every alternate adult individual chosen for anthropometry was covered for estimation of fasting blood glucose.

**Estimation of lipid profile:** Every fourth adult individual chosen for anthropometry was covered for estimation of lipid profile.

**Sampling procedures:** In order to cover representative samples for various investigations proposed in the study, the following sampling procedure was adopted:

#### Rural Areas

- From each of the five regions in India, *two states* were selected randomly
- From each selected state, *three districts* were selected randomly

- From each selected district, *two blocks* were randomly selected
- From each selected block, *five villages* were selected randomly
- From each village, *10 households* were selected randomly

Thus, a total of 600 households from 60 villages of 12 blocks from 6 districts of 2 states was covered in each of the five regions during each of the two major seasons and total 3000 households was covered in the rural areas.

**Selection of States:** In each region, two states representing different ecological zones was selected randomly. The details are provided in the below given table:

**Selection of Districts and Blocks:** In each state, three districts representing different geographical regions were selected randomly. In each of the selected district, two blocks were randomly selected. The details are provided in the below given table 38:

**Table 38. Selected regions, States and State Capitals for the Rural and Urban Surveys**

Regions	States	Capitals
North	Uttar Pradesh (UP), Punjab	Lucknow, Chandigarh
South	Andhra Pradesh (AP), Kerala	Hyderabad, Trivandrum
East	West Bengal (WB), Orissa	Kolkata, Bhubaneshwar
West	Maharashtra, Gujarat	Mumbai, Ahmedabad
North East (NE)	Arunachal Pradesh, Meghalaya	Itanagar, Shillong

**Selection of Villages:** In order to have spatial distribution, in each of the block, five villages were selected randomly (Fig 51).

**Selection of Households:** The entire village was divided into 5 geographical areas based on natural groups of houses, streets and mohallas by consulting village head.

From each selected village, 10 HHs were selected from five areas (group of HHs). For this purpose, the main village and its hamlets, if any was divided into 5 natural groups based on streets/*mohallas*/areas. It was ensured that at least one of the 5 areas will be inhabited by the Scheduled caste and or Scheduled tribe communities. From each of these areas, two HHs were covered by randomly selecting the first HH. Thus, a total of 300 households (10HHs x 30 villages) were covered in each State.

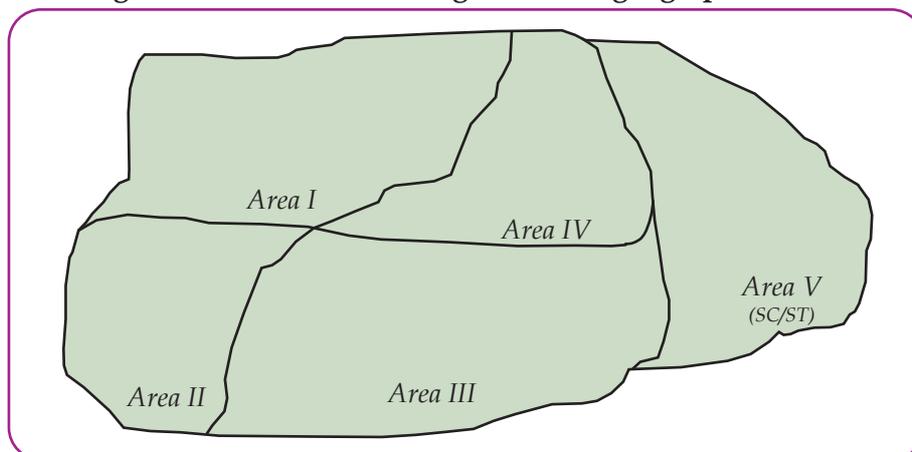
#### **Selection of random start in a geographical area**

In each geographical area, the HHs was numbered starting from northeast corner and continuing in a serpentine order. In the case of a large village, where the number of HHs in a geographical area was more than 100, each area was subdivided into two or more sub-areas, based on natural groups of HHs and from them one group was selected randomly for enumeration. The first HH was chosen by selecting random start using random number tables. In case, selected house was locked, next adjacent HH was covered for the survey. Similar procedure was adopted for covering HHs in all the remaining geographical areas.

Information pertaining to household demographic and socio-economic particulars and anthropometric measurements were carried out. Measurements of blood pressure was carried out among all the available adults in the selected HHs. Bio-chemical estimation for diabetes and lipid profile among sub sample of adult individuals in each selected village was also done.

**Urban Areas:** For the purpose of urban sample, the study was carried out in the capital cities of the selected 10 states. The survey was carried out in five socio-economic strata viz., High Income Group (HIG), Middle Income Group (MIG), Low Income Group (LIG), Slums and Industrial

Fig 51. Division of the village into five geographical areas



Laborers (IL). From each of these strata, 30 HHs were selected and covered for the survey. Thus, a total of 150 HHs (@ 30 HHs per socio-economic strata) was covered in each region. A total of 1500 households were covered in urban areas.

**Survey at two time points:** The survey was carried at two time points in the same states, districts, blocks and villages. Out of 10 HH to be covered for rural surveys, 5 HHs were covered in *first phase* and another 5 HHs in the *second phase*. Similarly in the Urban areas out of 30 HHs, 15 HHs in each socio economic group in the first phase and another 15 HHs were covered in the second phase.

**Investigations:** In each of the selected households, the following investigations were carried out by adopting standard procedures.

**Household Socio-economic and Demographic particulars:** Socioeconomic and demographic particulars such as age, gender, community, type of family, family size, occupation, income, literacy level of individuals and information about possession of agricultural land, type of crops raised and its production and livestock, type of dwelling, hygiene and sanitary conditions, etc. was collected in all the 10 HHs in each selected village.

**Frequency of consumption of various foods:** Frequency of consumption of various processed foods was collected from each of the individual present at the time of survey with the help of pre-tested and validated questionnaire.

**Food and nutrient intakes of individuals by 24-hour recall method of diet survey :** A 24-hour recall method of diet survey was carried out by standard procedure to assess the average daily food intakes of the individuals in every alternate HH covered for nutrition assessment.

**Anthropometry:** Anthropometric measurements like height, weight, mid upper arm circumference and fat fold thickness at 4 sites i.e. triceps, biceps, sub scapular and supra-iliac was carried out by using standard procedure and techniques in all the selected HHs. In addition waist and hip circumference was measured on all men and women of 18 years, except pregnant women, using standard techniques and procedures.

**Biochemical estimations:** Biochemical estimations such as fasting blood glucose and lipid profile was carried out on sub-sample of the subjects covered for anthropometry. The lipid profile included estimation of total cholesterol, serum triglycerides, low and high density lipoproteins (HDL) and were measured by using Cholitech equipment in the field itself.

**Fasting blood glucose levels:** Fasting blood glucose level was assessed from a finger prick blood samples collected from all the available adult men and women of  $\geq 18$  years in 10 HHs in the selected village, using one touch gluco-meter (ACCU CHEK, Active).

**Measurement of blood pressure:** Systolic and diastolic blood pressure was measured for 3 consecutive times, with 5 minutes interval between each reading in supine (lying down) position. It was measured among all the adults of 18 years covered for nutrition assessment by using mercury sphygmomanometer and adopting standard procedures.

**Estimation of Transfat:** The transfat was estimated in a sub-sample of processed foods likely to contain higher levels of transfat like cakes, chips, bread and biscuits, puff, snacks etc. were estimated by gas chromatography.

### **Survey instruments:**

The following are survey instruments that were administered for various investigations

- Household schedule
- Diet Survey schedule
- Compilation form
- Anthropometric schedule
- Food frequency schedule (Processed foods intake)

**Training and standardization of investigators:** The project staff were given orientation training and were standardized in all the methodologies, including the measurement of blood pressure before initiation of the survey, at NIN for a period of one week. During the training programme, the emphasis was given to achieve maximum intra and inter-individual agreement of the investigators with respect to all the measurements.

**Collaborating centers:** Five different collaborating Institutes/Centers were identified to execute the study in different regions of the country. While, NIN was the central coordinating Unit in addition to carrying survey in the southern region, four regional institutes / centers (either medical colleges or home science colleges or ICMR Institute / Center) were identified for carrying out this study in the North, East, North-East and Western regions.

The regional centers were given the responsibility of recruitment of the project staff, execution of field work, field supervision, quality control, data scrutiny and cleaning in their respective regions. The central coordinating unit was responsible for organizing centralized training cum standardization, data analysis, quality control and preparation of final report.

**Data collection:** Data collection was carried out simultaneously in 5 regions, North, South, East, West and North East, with the help of respective regional centers. Anthropometric measurements such as weight, height, waist and hip circumference were carried out by using standard procedures and techniques. For estimation of dietary consumption pattern, two methods were adopted viz., food frequency questionnaire was used to assess processed food consumption and a 24-hour recall method of diet survey to estimate food and nutrient intakes at individual level. Fasting blood glucose levels were estimated among rural and urban adults in a sub-sample by using glucometers (Accu-Chek Active, Roche diagnostics GmbH, Germany) and lipid profiles like total cholesterol, triglycerides, high & low density lipoproteins were estimated in the field itself by using Choletech equipment. Measurement of blood pressure was carried out using sphygmomanometer and 3 measurements were recorded with 5 minutes of interval from each of the adults. The data was collected in two spells to avoid seasonal variations, if any.

**Quality control:** The Scientists from NIN carried out periodic random checks of the data collection in the field. In addition, scientists from different regional centers in their respective states made periodical supervisory visits to check the quality of data collection and salvation of logistic problems, if any, faced by the survey teams.

**Ethical issues:** The study was approved by the Institutional Ethical Committee of National Institute of Nutrition (NIN), ICMR, Hyderabad and written individual consent was obtained from all the adults, who have undergone various biochemical investigations. Written informed consent was obtained from the head of the village and from the subjects.

**Data Analysis:** The data was scrutinized, cleaned and entered into the computers at the NIN, Hyderabad. The data was analyzed using SPSS version 17.0.

### Diet and Nutritional status

**Food and Nutrient Intakes of Individuals:** The average daily intake of different foods and nutrients by individuals was calculated according to different age and gender using 'Nutritive value of Indian foods' and nutrients were compared with recommended levels of ICMR (ICMR, 2010).

### ANTHROPOMETRY

**Infants and Preschool children:** The World Health Organization recommends use of SD classification (WHO 1983) to categorize the children into different grades of nutritional status. The percent distribution of under five year children according to underweight (weight for age), stunting (height for age) and wasting (weight for height) were computed using new WHO growth standards (WHO 2006) as given in table 39.

Table 39

SD Classification	Weight for age	Height for age	Weight for height
Median - 2SD	Normal	Normal	Normal
<Median - 2SD to Median -3SD	Moderate underweight	Moderate stunting	Moderate wasting
< Median-3 SD	Severe underweight	Severe stunting	Severe wasting

**School age Children and Adolescents:** The school age children and adolescents were categorized into various grades of nutritional status using the Tim Cole 2000, BMI age/sex centiles as given in table 40.

Table 40

Age/Sex Centiles	Nutritional grade
< 5 <sup>th</sup> centile	Undernutrition
5 <sup>th</sup> - < 85 <sup>th</sup> centile	Normal
85 <sup>th</sup> - < 95 <sup>th</sup> centile	Overweight
95 <sup>th</sup> centile	Obesity

### Adults

**Body Mass Index (BMI):** The adult men and women were categorized according to different grades of Body Mass Index (BMI) classification as follows:

$$\text{BMI} = \text{Weight (kg)} / [\text{Height (m)}]^2$$

**Waist Circumference:** All the adult men with waist circumference of 90 cm and women with 80 cm were identified as having abdominal obesity by using Asian cut-off levels.

**Waist-hip Ratio (WHR):** All the adult men with waist-hip ratio of 0.90 and women with 0.80 were identified as obese by using Asian cut-off levels (Table 41).

Table 41

BMI	Nutritional Grade	Classification
< 18.5	Underweight (Thinness)	James et al (1988)
18.5 - < 24.9	Normal	
25.0 - < 29.9	Overweight	
30 - 34.9	Obesity I	WHO (2000)
35 - 39.9	Obesity II	
40	Obesity III	
BMI		
< 18.5	Underweight (Thinness)	WHO/IASO/IOTF (2000)
18.5 - < 22.9	Normal	
23.0 - < 27.5	Overweight	
27.5	Obesity	

CED: Chronic Energy Deficiency

**Hypertension:** As per the definition of WHO, if the systolic blood pressure is 140 mmHg and/ or diastolic blood pressure is 90 mmHg, it is considered as hypertension (WHO/ISH 1999). As per JNC criteria VII, the classification of hypertension is given beside (Table 42).

**Table 42**

Blood Pressure	Systolic	Diastolic (mm Hg)
Normal	<120	<80
Pre-hypertension	120-140	80-90
Stage I hypertension	140-<160	90-100
Stage II hypertension	160	100

**Diabetes Mellitus:** The American Diabetic

Association (2004) has defined 'Diabetes' if the fasting blood glucose levels is 126 mg/dL and between 110 to <126 mg/dL is considered as impaired glucose tolerance (IGT).

## RESULTS

The present study was taken up with the objective to assess consumption of processed foods and non-processed foods in India. Food safety and nutrition are intimately related to human health. Undernutrition, overnutrition as well as consumption of unsafe foods are associated with non-communicable diseases and food borne infections. Food is exposed to contaminants during its transit through various stages in food chain and exposure to these chemical and biological contaminants pose health risk to humans. In order to ultimately carry out risk assessment, there is in need of food consumption pattern including actual intakes of processed and non processed foods by individuals. This report represents the first database in the country on consumption of processed and non processed foods.

Consumption of high energy dense foods, sugars and unhealthy fats are associated with age related diseases.

Therefore, as per the request of Food Safety and Standards Authority of India (FSSAI) and in view of the recommendation of the Joint Parliament Committee (JPC) to have information on food and nutrient consumption levels and pattern among urban and rural population in 5 geographical regions of the country i.e. North, South, East, West and North East, this study was planned at national level. It was a cross sectional and community based survey, adopting multistage random sampling procedure.

Information was collected on demographic and socioeconomic particulars of the households (HHs) and nutritional anthropometry, 24-hour recall method of diet survey and frequency of consumption of processed foods. Measurement of blood pressure, fasting blood glucose levels and estimation of blood lipids were carried out on sub-sample of subjects. Transfats were estimated in a representative food samples collected from all the regions. A total of 11,472 rural individuals of different ages from 2,900 HHs in about 300 villages were covered for anthropometry. Food and nutrient intakes and frequency of consumption of processed foods were collected from about 13,000 rural individuals. On a sub-sample of subjects ( $\geq 18$  years), measurement of blood pressures, estimation of fasting blood glucose levels and blood lipids were carried out. Similarly, a total of 5,017 urban individuals of different ages from 1,459 households were covered for the anthropometry and other investigations.

The analysis of data revealed that a majority of the HHs covered belonged to Hindu religion (59.8% and 70%) in rural and urban areas. About 35% and 49% of HHs belonged to other communities, while 29% and 19% belonged to Scheduled Tribes and 14% each belonged to Scheduled castes in rural and urban areas respectively. Nearly one-thirds of houses each were pucca, semi-pucca and kutcha in rural areas, whereas in urban areas, 64% of HHs were pucca. About 44% of the HHs had tube wells as source of drinking water in rural areas, while in urban areas, about 86% had access to tap water.

Cereals and millets formed the bulk of the rural and urban dietaries. Millets constituted only about 5% of the total cereals and millets consumed, and it was found in varying quantities

mostly in the West (Gujarat and Maharashtra). The consumption of all foods except roots and tubers was below the recommended levels of ICMR (1981) in all the age groups. The consumption of protective foods such as pulses, GLV, milk and fruits were grossly inadequate. Consequently, the intakes of micronutrients such as iron, vitamin A, riboflavin and folic acid were far below the recommended levels in all the age groups in both urban and rural areas.

Consumption of ready to eat fried foods were maximum in North (75%), followed by West (63%) and South & East (50% each), while ready to eat non-fried foods consumption was high in South (46%) compared to other regions in rural areas. Sweet items are also consumed avidly by all the people. However, maximum consumption of sweets was observed in North (71%), followed by West (59%) and other regions. The consumption of carbonated water beverage was highest in the West (28%), followed by South (26%). However, the mean intake was higher in South (23.6 ml/day/person) compared to West (8.69 ml/day/person) in rural areas.

In urban areas, consumption of ready-to-eat fried food ranges from 59% among HIG to 73% among IL and was higher in Northern region in all socio-economic groups (55 to 83%). Breakfast items were also consumed by about  $\frac{1}{4}$  of the population in all income groups and was higher in South region in all socio-economic groups (45-88%). Sweet items were consumed in all socio-economic groups and the consumption was maximum in North India in all socio-economic groups (65-84%). Consumption of carbonated beverages was higher among HIG and IL (35%) each.

In general, the prevalence of underweight, stunting and wasting among <5 year rural children was about 36%, 43% and 19% respectively, while it was 26%, 35% and 17% among urban under five year children. The proportion of undernutrition was higher among boys ( $p < 0.05$ ) compared to girls in rural and urban areas and it was higher at younger age group (1-3 years) compared to older age group (3-5 years) in both the urban and rural areas.

The prevalence of thinness among rural school children tended to decrease with increase of age from about 50% in 5-11 years age group to 44% among 12-17 year age group. Similarly, it was higher among urban school age children (40.5%) compared to adolescents (28.5%). The prevalence of overweight obesity was higher among urban adolescents (10.3%) compared to rural counterparts (4.3%).

At the aggregate level, the prevalence of chronic energy deficiency (BMI: <18.5) among rural men and women was 21% and 25% respectively, while it was only 10% each among urban men and women. However, the prevalence of overweight and obesity was 28% and 30% among rural men and women respectively, while it was higher among urban (49%) men and women (55%).

The prevalence of abdominal obesity among rural men and women was 14% and 27% respectively, while it was 24% and 48% among urban adult men and women respectively. The prevalence of hypercholesterolemia was higher among women (17.7% and 17%) as compared to men (12.1% and 11.4%) in rural and urban areas, while the prevalence of hyper triglyceredemia was higher among men (22-27%) compared to women (13-22%) in both rural and urban areas. The prevalence of diabetes was about 9% each among rural adults, while it was 10-13% among urban adults men and women respectively. However, the prevalence of impaired glucose tolerance (IGT) was higher among rural adults (13%) compared to urban adults (11%). The prevalence of diabetes was higher in Western region (17.4% and 14.8%) among rural men and women, while in urban areas, in South region, it was 18.3% and 16.5% among men and women respectively and was higher among HIG men (14.9%) and IL and Slum Dwellers (12% each) among women.

The overall prevalence of hypertension (SBP  $\geq 140$  and/or DBP  $\geq 90$ ) among rural men and women was 18% and 16% respectively, while it was higher among urban men (24.5%) and women (20%). The prevalence was higher in South and North East in rural and urban areas.

## CONCLUSIONS

The results indicated that the consumption of different food stuffs and nutrients among various age groups were below the recommended levels of ICMR. The consumption of processed foods was also considerably higher in some of the regions like West and Southern regions. The prevalence of undernutrition was higher among rural pre-school children as compared to urban children. The prevalence of overweight and obesity and non-communicable diseases was higher among urban adults as compared to rural. The prevalence of cardio-metabolic risk factors was higher among urban adults as compared to rural adults. In view of the above observations, it may be prudent to take steps to prevent and control double burden of diseases to limit its consequences in future life.

## 2 Effect of high fluoride and low calcium on bone metabolism in rats and genotoxicity

Fluorosis is a major public health problem all over the world. It is endemic over 25 countries across the globe and millions of people are affected by consumption of fluoride rich water due to lack of other alternate economically viable solutions. The problem of fluoride toxicity has been confirmed in 177 districts of various states of India as per the recent state of art report.

Studies carried out in different parts of the country and study conducted in Bihar by NIN showed that a high proportion of younger age groups are affected by fluorosis. Children of 2-3 years were found to be affected with severe form of crippling deformities of long bones of lower extremities due to fluoride toxicity. The crippling bone deformities were associated with poor socioeconomic status of the community, with low dietary calcium were found. The presence of signs of nutritional rickets among the preschool age children of the village along with typical bone changes in X-rays, indicate that the primary dietary deficiency of calcium or secondary deficiency of vitamin D, could be the causative factors along with fluoride toxicity.

Hence, a study was carried out to understand the interaction of calcium and fluoride in biological system in terms of nutritional status and skeletal metabolism as well as to study the effect of rehabilitation (providing normal calcium diet and fluoride free water) on reversal of fluorosis.

### AIMS AND OBJECTIVES

- To study the effect of low calcium in aggravation of skeletal fluorosis.
- To study the effect of rehabilitation (providing normal Calcium diet and fluoride free water) on reversal of skeletal fluorosis.

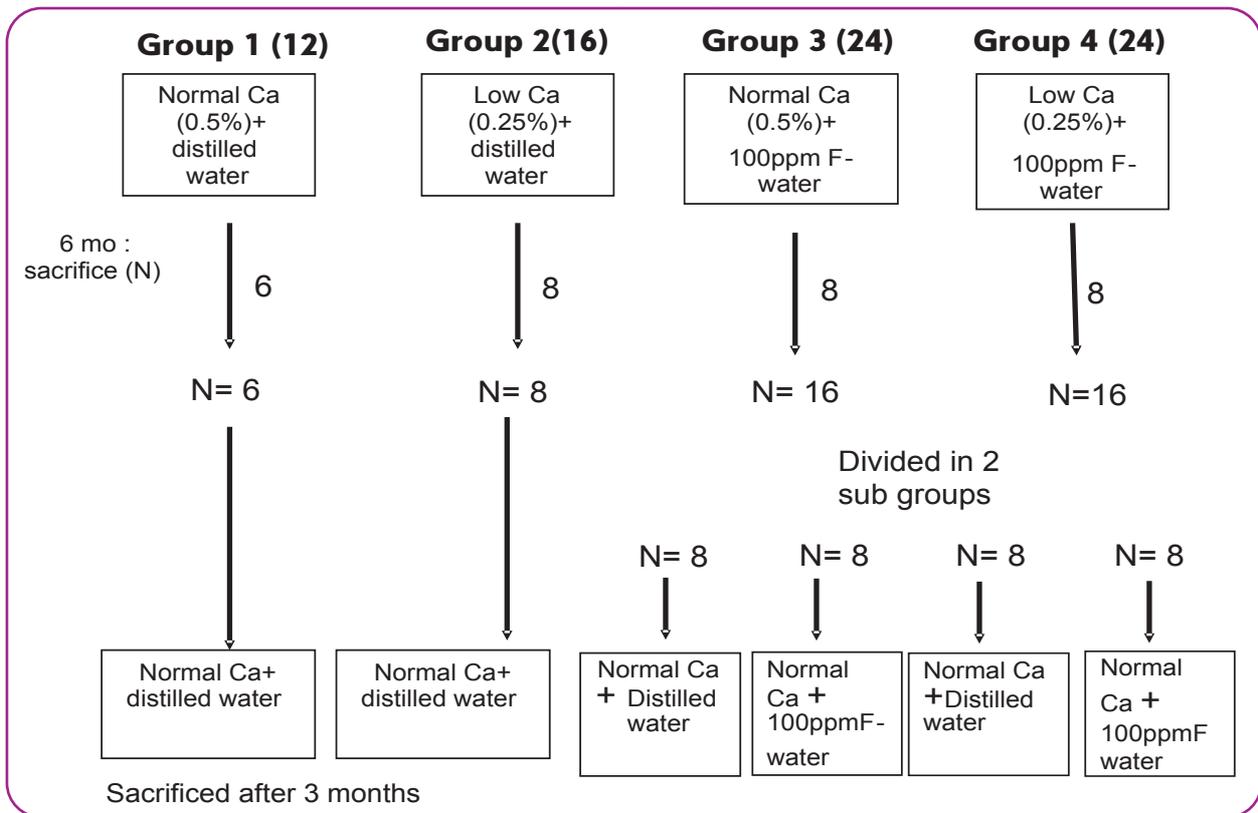
### METHODOLOGY

Body weight, food intake, water intake were measured and body weight gain, food efficiency ratio, calcium intake through diet and fluoride intake through water were calculated.

Urine and feces were collected by keeping the animals in metabolic cages for 3 consecutive days at the interval of 30 days. Calcium and fluoride in urine and feces were determined.

Blood was collected by retro orbital sinus puncture at an interval of 30 days. Serum was separated. Total acid phosphatase, tartrate resistant acid phosphatase, total alkaline phosphatase and bone specific alkaline phosphatase, calcium, phosphorus, 25 (OH)Vitamin D<sub>3</sub>, 1,25(OH)<sub>2</sub> vitamin D<sub>3</sub>, parathyroid hormone and osteocalcin were measured.

Study Design



DXA was done at the interval of 90 days.

Animals were killed by CO<sub>2</sub> asphyxiation. Femur bone was dissected out and cleaned of adherent tissues. Micro CT analysis, bone histomorphometry and biomechanical testing of femur were done at 180 days and 270 days. Bone fluoride and calcium were measured.

Histopathological examination of tissues was done.

**Gene expression studies:** Calcium sensing receptor, vitamin D receptor, Calbindin D 9k expression in duodenal mucosa and osteocalcin, osteopontin and osteonectin expression in bone was studied.

## RESULTS AND CONCLUSIONS

### Nutritional status

Food efficiency ratio of group 4 (0.25 % Ca+ F) was significantly lower in both phases of study and that was the reason that in spite of eating same amount of food (food intake was comparable in between the groups in both phases) body weight gain, lean body mass, fat and fat percentage of this group was significantly lower as compared to all other groups in phase I.

In phase II, lean body mass, fat and fat percentage of group 4 (0.25 % Ca+ F) was comparable with all other groups but body weight gain was significantly lower as compared to control.

### Calcium homeostasis

Serum Ca of fluoride treated groups (group 3 & 4) was significantly lower as compared to control at the end of phase I. In phase II, group 4a, which received both normal Ca diet as well as fluoride free water recovered better as compared to other subgroup (group 4b) of 0.25 % Ca+ F treated group (group 4). 25 (OH) Vitamin D<sub>3</sub> of 0.25 % Ca+ F treated group (group 4) was significantly lower as compared to other groups in first phase of study.

In calcium deficiency conversion of 1,25(OH)<sub>2</sub> Vitamin D<sub>3</sub> from 25 (OH) Vitamin D<sub>3</sub> increases that was the reason that 0.25 % Ca + F treated rats (group 4) showed significantly higher Serum 1,25(OH)<sub>2</sub> Vitamin D<sub>3</sub> as compared to all other groups in I phase of study.

In phase II, both 25 (OH) Vitamin D<sub>3</sub> and 1, 25(OH)<sub>2</sub> Vitamin D<sub>3</sub> were comparable in between the groups.

Serum parathyroid hormone (PTH) level was higher in groups 3 and 4 as compared to groups 1 in phase I. In phase II, group 4b which received 0.5 % Ca + F showed higher serum PTH level and the difference was significant as compared to group 3b (second subgroup of group 3, which received F). Urinary calcium excretion was lower in fluoride treated groups (group 3 and 4) as compared to control in phase I.

### Gene expression studies

Downregulation of any of the receptor studied (Calcium sensing receptor, vitamin D receptor, Calbindin D 9k) was not observed in presence of fluoride in phase I (fig 52). However, in phase II, Calb3 expression of fluoride treated subgroups 3b (2.5 fold) and 4b (2 fold) was down-regulated as compared to their FFW treated counterparts.

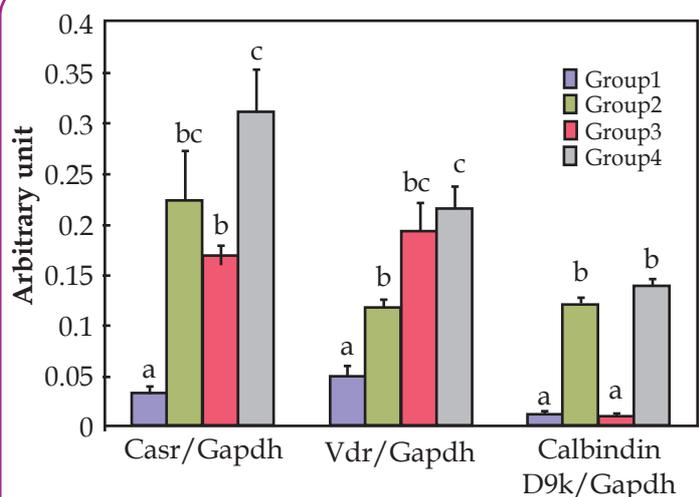
### Bone metabolism

There was significantly higher serum osteocalcin, % bone volume, bone surface density, trabecular number in fluoride treated rats (groups 3 and 4) as compared to their DIW treated counterparts (group 1 and group 2) in both phases of study which was further supported by increased bone formation rate as shown by bone histomorphometry. However, bone mineral content (BMC) and bone mineral density (BMD) of group 4 was significantly low as compared to group 1 and 3 in phase I. In phase II, also BMD and BMC of group 4 was low as compared to other groups but the difference was significant only for BMD. Biomechanical variables (Bone stiffness, Young's Modulus of bending, Flexural rigidity, Bone strength, Load at max, Max. Bending stress at break) were significantly low in group 4 as compared to other groups in both phases of study.

### Histopathological studies

Based on the histopathological observations, the various changes observed in all the three experimental groups were also seen in the control group. These changes did not appear to be related to restriction of calcium or increased fluoride intake at various time points and hence were not considered significant.

**Fig 52. Expression of Casr, Vdr and Calbindin D 9K normalized to Gapdh in duodenal mucosa of rats fed normal calcium (0.5%) diet/ low calcium (0.25 %) diet/ normal calcium (0.5%) diet + 100 ppm fluoride/ low calcium (0.25 %) diet+ 100 ppm fluoride for 6 months in phase I of the study.**



Bars represent Mean ± SEM, n=6 (group 1) or 8 (all other groups except group 1). Means without a common superscript are considered to be significant at P < 0.05. Abbreviations used: Casr, calcium sensing receptor; F-, fluoride; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; LCD, low (0.25%) calcium diet; NCD, normal (0.5%) calcium diet; Vdr, vitamin D receptor.

## CONCLUSIONS

- Nutritional status of low calcium and fluoride treated group was poor (in terms of body weight gain and body composition parameters) at the end of phase I.
- Observations indicated towards disturbance of calcium homeostasis in presence of fluoride in low as well as normal calcium treated rats.
- There was increased bone formation in presence of fluoride but quality of bone was poor in low calcium and fluoride treated group.

## EFFECT OF REVERSIBILITY

- Nutritional status and calcium homeostasis of rats normalized to some extent after providing normal Ca diet and fluoride free water for 3 months but not completely. Condition of subgroup 4a, which received normal calcium diet along with fluoride free water, was relatively better in terms of all variables reported as compared to the subgroup 4b, which received calcium along with fluoride water in Phase II.
- No improvement was observed in bone strength after providing normal calcium diet and fluoride free water for 3 months.
- Downregulation of Calb3 expression in fluoride treated subgroups at the end of phase II indicates that in the long term, fluoride toxicity may affect calcium absorption and thereby calcium homeostasis irrespective of nutritional background.

### 3 Kidney and bone disease – Role of silica, strontium and fluoride study in guinea pig

Strontium was first isolated 1808 and named after “Strontian” a Scottish town where it was found at high concentrations in calcareous rocks consisting of natural apatites in which strontium may occur at concentrations as high as 73 g/kg. It is high in ocean water up to 8 mg/l. Natural water sources, such as rivers, springs, and wells, contain smaller amounts of the element ranging from 0.021 mg/l up to 0.375 mg/l.

Strontium toxicity was focused mainly on radioactive Strontium -90 during that time the stable Srs' use was not in much practice. Subsequently it was started using for improvement in bone health and it could be known that the stable Sr replaces dietary Ca result in diminished growth, improper bone mineralization, and inhibit intestinal calcium absorption.

The recent study conducted in Uchapalli Village of Nellore District, A.P. to understand the etiology of kidney disease with osteosclerosis revealed that Silica (Si) and Strontium (Sr.) concentrations were very high in drinking water than municipal water supply of Hyderabad.

At present it is not known that whether silica and Sr in drinking water causes kidney damage and high bone density in human or whether only silica increasing bone density secondary to kidney damage at moderate level of fluoride in drinking water. To understand the mechanism of kidney damage in above situation, the present study was planned in Guinea pig.

#### AIM

To find out whether silica and Sr (with and without F) increases bone density secondary to kidney damage.

## OBJECTIVES

- To assess nutrition status
- To assess kidney function
- To assess bone formation and bone resorption
- Bone histomorphometry and bone density by DXA

## ANIMALS AND TREATMENTS

Forty eight, 2 month old male age matched guinea pigs (*Cavia porcellus*), weighing around 300 g were obtained from the National Center for Laboratory Animal Sciences (NCLAS) of the National Institute of Nutrition (NIN), Hyderabad, India and were randomly distributed into 8 groups of 6 each, first (control), second (fluoride treated), third (strontium treated) and fourth (F+Sr treated) group, which received distilled water, distilled water with 25 mg/L fluoride in the form of NaF, distilled water with 0.59% Sr in the form of  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$  and distilled water with 25 mg/L F + 0.59% Sr respectively. Standard guinea pig pellet diet, provided by NIN was fed ad libitum to all animals for duration of 6 months.

Body weights, diet and water intake of the animals were recorded at an interval of 15 days and 24 hrs urine was collected once in every 30 days for three consecutive days. Blood was drawn from retro-orbital sinus for every 2 months. The Sr concentration was analyzed using ICP-MS (Inductively coupled plasma-Mass Spectroscopy, Elan model 9000 from Perkin Elmer Sciex USA). Serum calcium, Magnesium, Copper and Zinc were analyzed by Atomic absorption spectrophotometer (Avanta, GBC, CAN 005472686, Australia) method. Urine was collected every month keeping animals in metabolic cages for 3 consecutive days and average was taken for urinary parameters.

The animals were sacrificed by CO<sub>2</sub> inhalation by keeping them in euthanasia chamber. All the vital organs such as kidney, liver, brain, heart, testis and mussel were collected; weights were recorded on digital weighing machine (Citizen, scale 0.1mg to 220g). Part of the organ was preserved using liquid nitrogen and some portion was preserved formalin to look into histopathological changes.

## RESULTS AND CONCLUSIONS

### *Nutritional status*

Diet intake and weight gain was reduced significantly in Sr, Si + Sr, F + Sr and F+Si+Sr groups from 120 day to 180 day as compared to control, Si and F.

### *Body composition*

- Total body fat and percent fat was significantly decreased in Sr, Si+ Sr, F + Sr and F+Si+Sr groups at both time points as compared to control, Si and F groups.
- Similarly lean body mass+BMC was significantly decreased in Sr group at 180 day as compared to fluoride group. However, it (lean body mass+BMC) was significantly decreased in F+Sr group as compared to control and F group at 90 day.
- Total fat as well as fat % was significantly lower in Sr and F+Sr groups as compared to control and F group at both 90 and 180 day. However, total fat and fat % was lowest in F+Sr group as compared to all other groups at 90 and 180 day.

### *Biochemical parameters*

- Serum concentration of Sr was significantly higher in Sr and F+Sr groups as compared to control and F groups. However, serum Sr concentration was significantly higher in F+Sr as compared to Sr.

- There was no significant difference in either blood urea or serum creatinine.
- Total Alkaline phosphatase (ALP) and bone specific ALP was significantly higher in F, F+Sr, Si+Sr and F+Si+Sr groups as compared to control, F, Si and F+Si groups and there was no significant difference in acid phosphatase and Tartarate resistant acid phosphatase (TRAP) at 2 and 4 month. However, total and bone specific ALP was significantly higher in Sr, Si+Sr and F+Si+Sr groups as compared to control, F, Si, F+Si and F+Sr groups at 6th month (Table 43).

### Organ-Body weight ratio

Brain and liver weight ratio was significantly higher in Sr, and F + Sr groups as compared to F group where as kidney weight ratio was significantly higher in Sr, F + Sr groups as compared to F group.

### Histopathology observations

Evaluation of all above mentioned organs showed no significant differences between the groups including controls except liver, testis and sciatic nerve. Evaluation of testis showed arrested spermatogenesis in the experimental groups and not in controls with the most in 16% of animals belonging to Sr and F+Sr (Fig 53).

### Bone micro-architecture

There was significantly low mineral apposition rate (MAR) and bone formation rate (BFR) in F+Sr group as compared to control.

### Micro CT analysis of femur and tibia

*Femur:* Trabecular volume and trabecular number was significantly higher in Sr group as compared to F and control and F group respectively. Whereas, trabecular thickness and trabecular

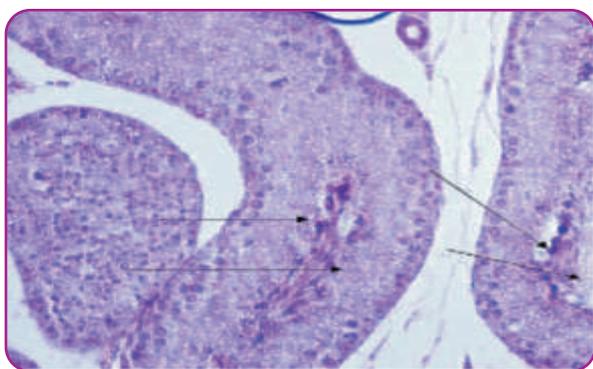
**Table 43. Body Composition by DXA (mean± SD) of control and experimental groups at 3rd and 6th month**

Group	Total Fat (g)		Lean Body Mass		Percent Fat	
	3 Month	6 Month	3 Month	6 Month	3 Month	6 Month
Control	137.2±41.80	125.0±34.10	737.4±89.23	795.4±131.78	15.5±3.18	13.7±3.69
Fluoride (F)	130.9±41.70	109.3±43.71	728.1±51.28	780.5±62.50	15.2±4.10	12.0±3.66
Strontium	47.64±26.33 <sup>a,b</sup>	36.0±28.60 <sup>a,b</sup>	616.7±105.51	617.0±133.36 <sup>b</sup>	6.8±2.83 <sup>a,b</sup>	5.0±3.37 <sup>a,b</sup>
Strontium+ F	24.0±27.20 <sup>a,b,c</sup>	24.5±23.22 <sup>a,b,c</sup>	452.4±243.90 <sup>abc</sup>	429.0±371.56 <sup>a,b</sup>	3.7±4.05 <sup>a,b,c</sup>	3.6±3.37 <sup>a,b,c</sup>

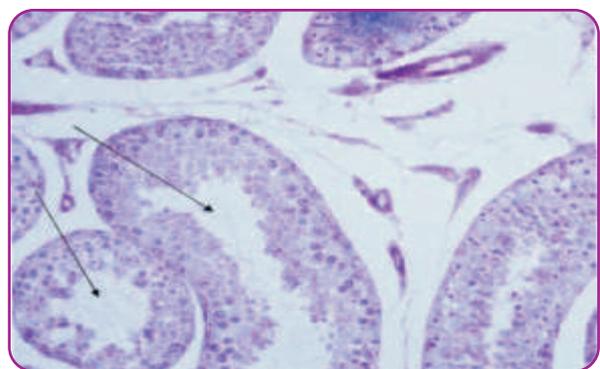
Means are considered to be different at P<0.05.

a - comparison with control, b - comparison with fluoride group, c - comparison with strontium group

**Fig 53. Testes: Arrested spermatogenesis was observed in Sr involved groups**



C/S of testes showing normal tubules with sperm presence in lumen (arrows) H& E X 200



C/S of testes showing tubules with no sperm presence in lumen (arrows) H& E X 200

separation was significantly higher in F group as compared to F+Sr and control, Sr and F+Sr groups respectively. SMI was significantly lower in Sr group as compared to F+Sr group.

**Tibia:** Trabecular thickness and trabecular separation were significantly higher as compared to Sr and, control and F+Sr groups where as trabecular number was significantly lower in F group as compared to control. Trabecular volume, trabecular number was significantly higher in F+Sr groups as compared to F and Sr group respectively. Whereas, SMI was significantly lower in F+Sr group as compared to control.

## CONCLUSION

Sr and Sr+F affects food intake and weight gain along with body composition and organ pathology. However, Sr+F group was more affected than Sr alone.

## 4 Assessing consumer behaviour and practice related to use of food labels in India

The production, sale and consumption of pre-packaged foods have witnessed a major surge in the recent years in India. Food labeling is one of the important population-based approaches that can help consumers make healthy food choices by providing the necessary nutrition information on the pack. The food label is one of the most important and direct means of communication of product information between buyers and sellers. Ideally, for consumers, food labels are tools to make informed and healthy choices. Food labels can also be viewed as potentially powerful tools of nutrition communication. In the Indian context, where overweight, obesity and the resultant non-communicable diseases are increasing; the effectiveness of food labels in discouraging consumption of unhealthy foods needs to be explored.

Packed foods hitherto sold in many Indian markets were only labeled with the product name, manufacturer's name and address, amount of product in the package, its ingredients and date of expiration. Recently, nutrient content declaration has been made mandatory on nearly all pre-packaged foods. Consumers also have more nutrition information due to expanded food labeling mandated by the Government of India. While there is no doubt that food labels will encourage healthy eating, there is increasing evidence from developed countries (where food labeling is more evolved) indicating that mere display of food labels cannot help the consumers make informed choices. However, there are scanty studies in India that looked in to the consumer knowledge, perceptions and practices pertaining to the use of food labels for making food choices. A recent study on 'the current scenario of food labeling in India', carried out by the National Institute of Nutrition (NIN) during 2008-09 with the support of WHO Country Office, India concluded that food labeling regulations in India are on par with those of the developed countries, but there are hardly any studies to examine the knowledge, practices and use of food labels by Indian consumers. The study also reiterated the need for nation-wide studies to understand the consumer knowledge and practices related to food labels for formulating strategies to make them user-friendly.

Therefore, the present study was carried out with the following objectives:

### OBJECTIVES

- To study the knowledge and perceptions related to food labels among consumers of different age groups (adolescents, adults and older population) from different economic groups in metro cities.

- To examine practices related to use of food labels while buying pre-packaged foods at market sites, and
- To develop an instrument for assessing food labels for both regulators and consumers.

## METHODOLOGY

**Study design:** A cross-sectional consumer market survey was carried out using stratified random sampling technique.

**Subjects:** The subjects were drawn from different age groups, namely, adolescents (10-19 years), adults (20-59 years) and elderly population ( $\geq 60$  years) for the present study.

**Study location:** The study was carried out in 2 metro-cities, one in North, New Delhi and another one in South India, Hyderabad. Each metro was divided into four regions – North, South, East and West.

**Investigations and Data Collection:** Both the quantitative and qualitative research methods were used for data collection.

**Quantitative data collection:** For obtaining the quantitative data, a pre-tested questionnaire was administered on consumers as part of intercept surveys conducted at exit points of randomly selected super-market sites. This questionnaire elicited information from respondents on demographic characteristics (gender, age, education, profession, family type etc) as well as data on important considerations while buying packed foods like frequency of checking labels; information they get from labels etc. This information was triangulated with qualitative data collected by conducting Focus Group Discussions (FGDs).

**Sample for quantitative data collection:** Assuming that 25% of the consumers buying packed foods check food labels, taking 95% confidence interval and 20% relative precision, the sample number arrived at was 300 per each age group making the total sample 900 (@300 x 3 age bands) from each metro city. The total number of subjects for the study therefore was to be 1800 (@900 per city x 2 metros).

In order to select the required sample of 900 subjects/city, each metro city was divided into four natural strata and 300 in each age group (@75 subjects x 4 strata) were selected from 20 randomly selected super markets.

**Qualitative data collection:** About 3 FGDs were conducted in each age band with the respondents in every city making the total of 9 FGDs per city.

**Study setting and Respondents for qualitative data collection:** In Hyderabad, 11 FGDs were conducted each from the 3 age groups of the 4 natural zones. Care was taken not to include those who had participated in the questionnaire survey. The homogeneity of the participants in the FGDs was limited to the facts that they all buy pre-packaged foods and belong to a particular age group. Gender homogeneousness was considered only among the adult and elderly groups where as among adolescents mixed groups were taken.

**Preparation of theme guides/Discussion points:** A theme guide, which listed the themes/topics around which the discussion would focus, was used. The specific questions were derived from the survey questionnaire. The probes were used to extract all the related information covered in the survey questionnaire.

**Conducting FGDs:** The team consisted of a moderator, two note takers (who were trained to conduct FGDs in a standardized way) and sometimes a photographer. The FGDs were conducted at the locations convenient to the participants. The recorded discussions were transcribed on the day after the discussion by the note takers using the notes, supplemented by the audio-visual recordings especially when more than one person had spoken at the same time.

## DATA ANALYSES

The quantitative data was scrutinized and checked for consistency and entered into the computers. The SPSS window version 17.00 was used for analysis of data. Appropriate statistical methods were used in the data analysis.

From the qualitative data, individual FGD reports were compiled with the help of the respective moderators by one of the investigators. In compiling the individual reports, raw data were organized into codes (according to the method suggested by Krueger (1998) and Newman (1994)) based on the themes. Then separate documents were produced for each state by including the comments of the girls from the respective groups in response to the themes raised during the discussions. These reports were read independently by two other investigators who agreed on the interpretations at both the stages. All these individual reports were in turn compiled, and similar findings were grouped under each theme with relevant comments to present the results.

## RESULTS

### Salient Findings

For collecting the quantitative data, 1832 intercept surveys were carried out at supermarket sites using structured questionnaires. In order to triangulate this information, 21 Focus Group Discussions (FGDs) were conducted in all the three age groups in both the cities. About 45% of the consumers across the age groups reported that they bought packed foods once in a week and about a fifth of respondents bought these foods every day. Consumers across the age groups buy packed foods as they consider that they are tasty and their quality and quantity is ensured, while convenience and ease of use were reported as the important considerations in the qualitative data. Although 90% of the consumers across the age groups reported that they check food labels most of them (81%) cited safety as an important concern for doing so. Majority of them looked for manufacturing date, expiry/best-before date.

It was observed that only about 1/3<sup>rd</sup> of the consumers checked nutrition information and list of ingredients. The reason cited for not checking the nutrient information was that the information was 'too technical to understand' and some of the respondents in the qualitative study informed that the lack of nutrition knowledge as an important reason for not checking the nutrition information on labels. However, it was observed in the FGDs that women and adolescent girls who were concerned with 'fat' and 'sugar' intake were in the habit of checking the nutrition facts.

A significantly greater number of consumers with higher education qualifications were checking the nutrition information. Only about 60% of the respondents checked the quality symbols, with more of the respondents with higher education checking them more often. In the quantitative data more number of elderly population (than the other groups) reported to have checked the quality symbols, interestingly, hardly any of the participants of that age group in the FGDs knew about these symbols. There was also a felt need to educate consumers on various aspects of food labels. Some of them also suggested that 'symbols' or hologram based food labels could be experimented to indicate 'healthy' or 'unhealthy' packed foods.

The earlier study conducted on current practices of food labeling had indicated that the compliance with regulatory requirements is almost 90%, however, there is no specific check list for grass root level food regulators to check the compliance. And the current study had indicated that there is an immediate need to take up educational activities for consumers to help them understand what goes into food labeling. In accordance with these findings, a check list for the food safety regulators is prepared for quick assessment of food label for regulatory compliance. In order to educate the consumers on what to look on the label before choosing food product, a checklist for consumers is also prepared.

Worldwide acute diarrheal illness accounts for an estimated 2.5 million childhood deaths annually. WHO defines diarrhea as the passage of loose or watery stools at least three times in a 24hr period. Acute diarrheas, the most usual form of diarrheal illness, have an abrupt onset, resolve within 14 days, and are mostly caused by infections. Globally acute diarrheal diseases constitute 17% of mortality among children under 5 years of age (WHO 2003). The World Health Organization (WHO), in conjunction with other national public health agencies, is coordinating a number of international activities designed to assist countries in the strengthening of disease surveillance and to determine the burden of acute gastroenteritis. The incidence of bacterial enteric infection among the children under 5 years of age was found to be the highest for *Campylobacter*, *Salmonella* and *Shigella*. The most common bacterial causes of diarrheal illness among children are *Campylobacter* Spp., *Salmonella* Spp., *Shigella* Spp and Shiga toxin producing *Escherichia coli*. In India, an estimated 4 lakh children below the age of five years die each year due to diarrhea. The bacterium responsible for 50% of the cases of persistent diarrhea in India has been discovered at AIIMS and named as *Enteroaggregative E.coli*. *Rotavirus* was demonstrated to be the major cause of diarrheal disease at AIIMS (AIIMS, 1994). The reported cases and deaths due to acute diarrheal diseases in India during 2004 are 9575112 and 2855 (Health Info of India, 2004). Every year there is an increase in the reported cases of acute diarrheal diseases in India. Over a period of time there is a change in the profile of etiology of acute diarrhea so there is a need to isolate, identify and characterize enteric pathogens in pediatric population and factors associated with their occurrence. Antimicrobial resistance among enteric pathogens in developing countries is a critical area of public health concern. The usual causative agents of diarrhea, *Shigella*, *Campylobacter* and *Salmonella* are becoming increasingly resistant to most agents commonly in use. The IAP task force recommends that, depending upon the sensitivity pattern of the area, cotrimoxazole and fluoroquinolones should be the antibiotics used to treat acute dysentery. Indiscriminate use of antibiotics has caused increasing resistance to commonly used antibiotics. Scarcity of data, on the resistance pattern from most parts of the country is the major challenge in deciding the recommendations. So there is a need to do antibiotic sensitivity assay which will provide early warnings of the emergence of resistant bacterial strains.

### OBJECTIVES

- To isolate, identify and characterize the pathogens from pediatric diarrheal infections
- To screen the isolates of enteric pathogens for their antibiotic sensitivity

### METHODOLOGY

#### *Enrollment of subjects*

Children in the age group of 6 months to 5 years who are suffering from acute diarrhea and attending outpatient clinic or admitted in Niloufer Hospital, Hyderabad were enrolled for the study. A standard questionnaire was administered to the parent or the guardian to elicit information on age, sex, symptoms, onset of disease and treatment, time of initial symptom, medical history and laboratory findings.

#### *Sample collection*

Stool specimens were collected from the children with diarrhea under 5 years of age referring to Niloufer Hospital for a period of one year. The samples were inoculated into carry Blair transport medium during transportation to the laboratory.

### Sample analysis

The specimens were checked microscopically (Direct smear, Gram staining) then each specimen were cultured according to the standard method. In order to evaluate the role of major bacterial pathogens, all specimens were cultured using xylose lysine deoxycholate agar (*Salmonella* and *Shigella*), Yersinia selective agar (*Yersinia enterocolitica*), Campylobacter selective agar (*Campylobacter*), Listeria selective agar (*Listeria monocytogenes*) and Macconkey Agar (*E.coli*). The isolates were identified and confirmed by biochemical tests and Real Time-Polymerase chain reaction (RT-PCR).

For isolation of *Salmonella* Spp the specimen was inoculated on Selenite F Broth for 12 hrs before primary culture. For isolation of *Campylobacter* Spp which has microaerophilic requirements, the gas pack system and anaerobic jar was used. After inoculation the plates were incubated at 42° C for 48 hrs. Identification of suspicious colonies were carried out according to standard criteria. For *Rotavirus* identification, ELISA Test Kit was used.

### RESULTS

1. A total of 748 mothers or guardians who brought their children (6 months – 5 yrs of age) to Niloufer hospital for treatment of diarrhea were administered a pre-tested questionnaire to collect the background information. Stool samples could only be collected from 502 children (Male-60.3%, Female-39.7%).
2. A total of 632 cultures were isolated from 502 stool samples. More than 54% of children were below one year of age and only 3% belonged to more than 4 years of age.
3. Major symptoms reported were diarrhea, fever and vomiting. In 10% of the households, other members of the family had similar symptoms, in 9% cases the children had attended large gathering along with their mothers within previous seven days. For 81% of children, Municipal tap water was the source of drinking water and 65% of children were consuming complementary foods.
4. Examination of the stool samples of 502 children under 5 years of age showed that 367 (73%) children were harboring one or more of the 7 major bacterial pathogens, i.e, *Escherichia coli* (36.2%), *V.cholerae* (14.5%), *V.parahaemolyticus* (0.9%), *Salmonella* spp. (18%), *Shigella* (8.3%), *Campylobacter* spp. (14.2%) and *Yersinia* (3.3%). A total of 81 stool samples were analysed for *Rotavirus*. About 27 (33.3%) samples were positive for *Rotavirus*.
5. Examination of stool samples from 502 children under 5 years of age indicated that 229 (45.6%) of children harbored one or more of the *E.coli* serotypes. Among 229 serotypes, 61 cultures were characterized for *E.coli* serotypes.
6. Among the 61 strains of *E.coli*, Enteropathogenic *E.coli* (EPEC) accounted for 41%, Enterotoxigenic *E.coli* (ETEC) 13.1%, Shigella toxigenic *E.coli* (STEC) 34.4% and *E.coli* 0157:H7 27.8%. Antibiotic sensitivity assay indicated that more than 70% of the *E.coli* isolates were resistant to Norflaxacin, Amoxycillin, Co-Trimoxazole, Ampicillin, Ceftriaxone, Cefotaxime and Metranidazole (n=86).
7. The isolation rate was much greater in males than in females, and frequency of the bacterial species isolated varied according to the age group. For example, 46.3% of *Escherichia coli* isolates were from children under 1 year old. The prevalence of bacterial pathogens was higher in children below 1 year age group than the other age groups (Table 44).
8. Two species of Vibrio were identified in our study, *V.cholerae* and *V.parahaemolyticus* with *V.cholerae* being the major isolate. *Escherichia coli* was the most common (36.2%) etiologic agent detected among the children with diarrhea, followed by Rotavirus (33.3%) and other bacterial pathogens.

**Table 44. Age distribution of positive isolates with gastrointestinal infection**

Organisms	Age (Years)										Total	
	<1		1 to <2		2 to <3		3 to <4		4 to <5		No	%
	No	%	No	%	No	%	No	%	No	%		
<i>E.coli</i>	106	46.3	68	29.7	33	14.4	9	3.9	13	5.7	229	100
<i>V.cholera</i>	34	37	18	19.6	16	17.4	12	13	12	13	92	100
<i>V.parahaemolyticus</i>	2	33.3	2	33.3	1	16.7	0	0	1	16.7	6	100
<i>Salmonella</i>	59	51.8	32	28.1	10	8.8	8	7	5	4.4	114	100
<i>Shigella</i>	24	45.3	13	24.5	6	11.3	3	5.7	7	13.2	53	100
<i>Campylobacter</i>	39	43.3	27	30	8	8.9	7	7.8	9	10	90	100
<i>Yersinia</i>	9	42.9	5	23.8	1	4.8	3	14.3	3	14.3	21	100
<i>Rotavirus</i>	10	37	9	33	5	18.5	2	7.4	1	3.7	27	100

\*632 culture positive isolates from 502 children

- Seasonal distribution of the enteropathogens in stool cultures indicated that the prevalence of the bacterial pathogens was higher during summer (45.5%) followed by rainy (31.6%) and winter season (22.7%) (Table 45).
- Antibiotic sensitivity assay showed that more than 70% of the *E.coli* isolates were resistant to Norfloxacin, Amoxicillin, Co-Trimoxazole, Ampicillin, Ceftriaxone, Cefotaxime and Metronidazole. More than 70% of the *Salmonella* isolates were resistant to Amoxicillin, Co-trimoxazole, Ampicillin, and Metronidazole and 65% of the isolates were resistant to Ceftriaxone, Cefotaxime and Metronidazole. More than 50% of the *Vibrio* spp. isolates were resistant to Amoxicillin, Co-Trimoxazole, Ampicillin, and Metronidazole and 30% of the isolates were resistant to Ceftriaxone, Cefotaxime and Metronidazole.

**Table 45. Antimicrobial resistance of enteric bacterial isolates from children with acute diarrhea in Hyderabad, India**

Organisms	Number of isolates resistant to antibiotics, (%)									
	Antibiotics									
	G	NX	AK	AM	COT	A	CTR	CTX	MT	FR
<i>E.Coli</i>	21 (24.4)	62 (72)	17 (20)	81 (94.2)	70 (81)	78 (91)	14 (70)	16 (80)	18 (90)	9 (11)
<i>Salmonella</i> <i>spp.</i>	13 (19.4)	29 (66)	11 (16.4)	38 (86.4)	48 (72)	5 (88)	16 (69)	15 (65)	22 (96)	9 (13.4)
<i>Vibrio</i> <i>spp.</i>	9 (24.3)	12 (35.3)	9 (24.3)	17 (50)	19 (51)	25 (68)	2 (33)	2 (33)	5 (83.1)	8 (22)
<i>Shigella</i> <i>spp.</i>	4 (33.3)	5 (62.5)	2 (17)	4 (88)	7 (58)	7 (58)	2 (50)	1 (25)	4 (100)	1 (8.3)
<i>Yersinia</i> <i>spp.</i>	2 (22.2)	5 (62.5)	2 (22.2)	7 (88)	7 (78)	9 (100)	1 (100)	1 (100)	1 (100)	3 (33.3)

## CONCLUSIONS

- E.coli* was found to be the major bacterial pathogen causing diarrhea among children.
- Emerging bacterial pathogen like *E.coli* 0157:H7 was isolated from diarrheal patients during the study.

3. In general *E.coli* serotypes were resistant to Ampicillin, Amoxicillin, Metranidazole and Co-Trimoxazole. Further, study on the prevalence of other serotypes of *Escherichia coli* and their antibiotic resistance among diarrheal patients is required.
4. The patterns of resistance to common antimicrobial agents in Hyderabad indicated that designing a surveillance system for antimicrobial resistance and introduction of integrated guidelines for the appropriate use of antibiotics are needed.

6

### Study on determination of levels of aflatoxins in stored paddy and rice of PAU 201 variety collected from 6 districts of Punjab

The present study is based on an investigation was carried out by ICMR on the fungal and aflatoxin contamination of PAU-201 rice variety developed by Punjab Agricultural University, Ludhiana from various districts in Punjab during August to September 2010. The PAU201 rice variety is a semi-dwarf high yielding, disease resistant, short duration rice variety requiring less amount of irrigation water compared to other varieties that was released by the PAU in the *Kharif* season of 2007. The PAU 201 variety rice was not permitted for milling and subsequent distribution due to presence of damaged grains at levels exceeding the PFA acceptable limits of 5% and FCI limits for pin point damaged grains of 1%. As a result 30,000 tonnes of the rice was held up in storage in rice mills and FCI godowns from public distribution and became a subject of considerable safety and quality concern to the GOI. An emergency survey was undertaken by ICMR at the request of Ministry of Health GOI during August 2010 to September 2010 to assess the extent of fungal and aflatoxin contamination in the stored PAU 201rice in different districts of Punjab so as to consider any health hazard before releasing the rice to the population.

#### AIMS AND OBJECTIVES

- i) To assess the extent of fungal and aflatoxin contamination in the PAU 201 rice stored in various rice mills and godowns in Punjab.
- ii) To assess the percentage of damaged grains consisting of black tip, pin point and other discoloured grains.
- iii) To assess whether the black coloured and pin point damaged grains contain iron.

#### Work done during the year

##### *Collection of PAU 201 rice samples from Punjab:*

Samples were collected from 35 locations in six districts of Punjab namely Barnala, Bathinda, Firozpur, Mansa, Moga and Muktsar on the basis of availability of PAU201 stocks in these mills (Table 46). Sample collection was done from 21<sup>st</sup> to 23<sup>rd</sup> August, 2010. A total of 35 paddy samples of PAU 201 variety were collected from the 35 locations from the six districts. Each of these 35 locations had a stock of more than 30 thousand bags of PAU-201 variety of paddy. These bags were stored in 5-15 stacks. Rice breeders from PAU, Ludhiana helped to identify PAU-201 variety as bags containing different varieties were stored together and also at times there were different varieties present within the same bag. In addition to paddy samples 11 damaged rice samples containing discoloured, mould and insect damaged grains that were segregated by a sortex machine in the mills where available were also collected for aflatoxin analysis.

Table 46. Details of samples collected

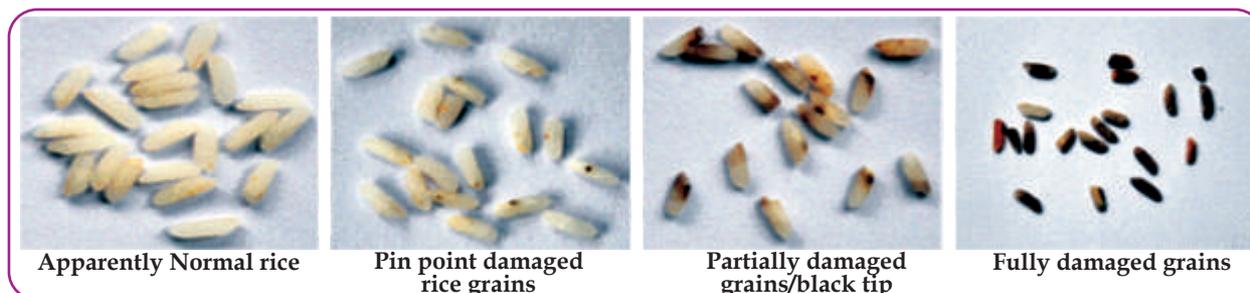
S.No.	District	Location	Sample collected	
			Sample	Number
1	Barnala	Thuliwal	Paddy Sorted damaged rice	1 1
2	Batinda	Batinda, Rampura, Bhucho	Paddy Sorted damaged rice	8 2
3	Mansa	Bhikhi, BiroKe kala, Budlada, Khurd Road, Barnal Road, Talbandi Road, FCI Depot, Ubhah, Burj Chhabar Road, Akalia	Paddy Sorted damaged rice	13 8
4	Muktsar	Giderwaha	Paddy	6
5	Faridkot	Jaitu, Faridpur Road Hasipul	Paddy	5
6	Mogha	Kot Ise Khan	Paddy	2
Total	Paddy			35
	Sorted damaged rice			11

**Sampling procedure:** Rice samples (as paddy and milled rice) were collected as per procedures recommended by PFA. The quantity of paddy stored at each location ranged from 27,000 to 1,60,000 bags each bag consisting of 35Kg paddy. Bags were randomly chosen for collection of samples which were drawn at the periphery and top core of the stacks. Samples were drawn from selected bags with the help of sampling spear until an aggregate sample of 15-20 kg sample was collected. The paddy samples were thoroughly mixed on a flat platform and 7-10 kg of sample was weighed on a weighing machine and transferred to polybags, labeled and sealed and transported to the analytical laboratory for analysis of aflatoxins.

**Sub sampling of samples for analytical work:** The paddy samples weighing 7-10kgs each were sampled individually using method of quartering until 1kg sample was obtained. Each of the 35 analytical samples obtained were milled and polished in a laboratory mill at the Directorate of Rice Research, ICAR, GOI Hyderabad. About 150g of milled rice was homogenized in a laboratory mill and 50g sample was used for aflatoxin analysis. Damaged rice samples weighing one kg each obtained from the Sortex machine at the mills was sampled by quartering to get a final sample of 100 g of rice for aflatoxin analysis.

**Estimation of percentage of damaged grains:** A sub sample of 20g milled rice was used for segregating damaged grains into black tip, pin point, fully damaged and other discoloured grains (Fig. 54) and calculating percentage of damaged grains as per BIS protocols.

Fig 54. Gross appearance of normal and damaged PAU 201 rice grains



**Analysis of aflatoxins:** Aflatoxins were analysed by HPLC method as per protocol of AOAC Official Method 990.33 using silica gel chromatography and precolumn derivatization with trifluoroacetic acid and a limit of quantification (LOQ) of 5µg/kg. Briefly aflatoxins were extracted with methanol-0.1N HCL (v/v), purified on silica gel (60-120mesh) column and evaporated on steam bath. The residue was reconstituted in acetonitrile-water (1:9 v/v), derivatized with trifluoroacetic acid and detected on HPLC using water acetonitrile-methanol-tetrahydrofuran (55:30:15:1) as the mobile phase at a flow rate of 1ml/min. HPLC system consisted of Agilent HP 1100 Series with HP- CHEM STATION (Rev A 07.1(682) software, G1321 fluorescence detector G1311 quaternary pump, and G1322 Degasser. The HPLC column consisted of Waters Spherisorb ODS-2, 4.6 x 250mm, 10µm particle size. Detection was achieved at Excitation and Emission wavelengths of 365nm and 440nm respectively. Aflatoxin B<sub>1</sub> and B<sub>2</sub> in samples were quantified from peak areas of sample and pure aflatoxin B<sub>1</sub> and B<sub>2</sub> standards (Sigma. Co.A6636 and A9887).

**Estimation of aflatoxin levels in rice at different limits of damaged grains:** To assess the extent of aflatoxin contamination in rice in the presence of damaged grains, the aflatoxin levels estimated in the sorted damaged rice samples were extrapolated using cut off levels of damaged grains at 3, 4.75, 10 and 12.3% on the basis of FCI specifications and the levels of damaged grains observed in the current study.

**Scanning Electron Microscope Study for detection of fungal growth:** Scanning Electron Microscopy studies were performed on milled and polished rice samples as well as sorted damaged rice samples after segregating into apparently normal appearing rice, pin head damaged rice and partially and fully damaged rice. Scanning Electron Microscope (Hitachi S-3400N) was used to study presence of fungus on the surface as well as in the transverse sections of the rice grains. Grains were cleaned to remove dust and other visible particles and coated with Gold (600 A°) using E-1010 Sputter coating unit and studied at 15 KV. Pictures were captured using Scanning Electron Microscope (Hitachi S-3400N) from 50X to 7500X magnification.

#### **Detecting presence of iron in black tipped/pin point damaged rice grains**

**Prussian Blue Staining Method:** The test is based on the formation of a bright blue pigment called Prussian blue, or ferric ferrocyanide in the presence of any ferric ion<sup>(+3)</sup> in the tissue/grains. The method involved suspension of 5-10 rice grains in 2mL of Prussian blue staining reagent and incubation for 10 minutes at room temperature. The stain was subsequently removed by washing the grains thrice with 0.1N HCl and placed on a white paper and photographed. Prussian Blue staining was performed on normal, black tipped, pin point damaged, partially damaged and fully damaged PAU201 rice. As controls local market rice was used and also ultra rice which was reconstituted rice made with rice flour fortified with iron.

**Energy Dispersive X-ray (EDX) analysis:** The technique was used for identifying the elemental composition of the specimen, or an area of interest thereof. The samples were placed on SEM stub with double sided adhesive tape and bombarded with an electron beam inside the SEM to study the elemental composition of the rice samples.

## **RESULTS**

**Milling of PAU 201 rice:** The mean moisture content of paddy before milling was 14.2±0.49 %. An average total yield of 68.99±4.71, with mean head rice of 34±7.4% (range 23 to 52%) and broken of 35.96±7.57% (range 23-64%) were obtained. The weight of head rice obtained ranged from minimum of 137 to maximum of 316g/1000g of paddy milled.

**Aflatoxin contamination in PAU 201 rice samples:** Out of the 35 milled paddy samples analyzed, none of the samples was found to be contaminated with aflatoxin levels above the PFA, GOI limit of 30µg/kg (Table 47). The level of aflatoxin was below the limit of quantification of 5µg/kg in

Table 47. Results of aflatoxin\* analysis of paddy and damaged rice samples

Sample	Total No. analysed	No. of samples Aflatoxin levels ( $\mu\text{g}/\text{kg}$ )				
		BLOQ**	<15	15-30	>30	Range
Paddy	35	32	2	1	Nil	6-26.2
Damaged rice	11	1	2	1	7	7.7-151.3

\*Aflatoxin B<sub>1</sub> & B<sub>2</sub> \*\*Below Limit of Quantification:5 $\mu\text{g}/\text{kg}$

91.4% of the samples analysed. Analysis of the sorted damaged rice samples indicated that 64% of the samples had aflatoxin levels exceeding the tolerance limits and ranged from 8 to 151 $\mu\text{g}/\text{kg}$ .

**Presence of damaged grains in rice:** The percent of damaged grains from the rice milled from 35 paddy samples ranged from 3.1% to 12.25% (Table 48). Around 14% of the samples had below 5% damage, as per PFA criteria with a mean percent damage of 4.05, while majority (85.7%) of the samples indicated presence of damaged grains >5% with a mean percent damage of 8. The range of pin head damage in 35 samples was from 0.47% to 2.53%. Pin point damaged grains were below the FCI specification of 1% in 34.3% of the samples with a mean of 0.8% whereas about 66% of the samples exceeded the 1% limit with a mean of 1.43%. Without including pin point damage the percentage of samples with <5% and >5% damage was found to be 40% and 60% respectively (Table 48). No correlation was found between percent damage and aflatoxin levels.

**Estimation of aflatoxin levels in rice at different limits of damaged grains:** When aflatoxin levels in sorted damaged rice samples were extrapolated at 3.0, 4.75, 10 and 12.3% damage cut offs the level of aflatoxin in rice was below the tolerance limits (Table 49). No correlation was observed between the percentage of damaged grains and aflatoxin levels.

**SEM studies on fungal contamination in rice samples:** SEM studies performed in different categories of milled rice samples indicated that there was no evidence of fungal presence on surface or at a deeper plane in milled paddy samples or in the pin head and partially damaged rice samples (Fig 55,56). Presence of fungal structures with spores was seen only in the fully damaged rice samples (Fig 57). Presence of iron in discoloured rice: Prussian Blue staining revealed that except for the Ultra rice (fortified with iron), none of the other samples stained blue thus indicating that the black spots of PAU-201 variety are not due to presence of iron (Fig 58). EDX analysis of pin point damaged grains also did not indicate presence of iron (Table 50).

Table 48. Level of Damaged grains in PAU201 paddy samples

No. of samples	Damaged grains (%)					
	<5 (Total)	>5 (Total)	<1ppd*	>1ppd	<5(w/o ppd)	>5(w/o ppd)
	5	30	12	23	14	21
%	14.3	85.7	34.3	65.7	40	60
Mean %damage	4.05 $\pm$ 0.69	8.02 $\pm$ 2.03	0.8 $\pm$ 0.17	1.43 $\pm$ 0.4	4.17 $\pm$ 0.75	7.68 $\pm$ 1.59
Range %damage	3.1-4.65	5.15-12.3	0.47-0.98	1-2.53	2.6-4.9	5.2-10.4
Weight of damaged grains(g) (range)	0.62-0.93	1.03-2.45	0.47-1.0	1.02-2.53	0.62-1.24	1.4-2.45
Mean $\pm$ SD (g)	0.81 $\pm$ 0.14	1.6 $\pm$ 0.41	1.3 $\pm$ 0.5	1.61 $\pm$ 0.42	1.02 $\pm$ 0.188	1.8 $\pm$ 0.32

\*ppd: pin point damaged grains only

Table 49. Aflatoxin levels ( $\mu\text{g}/\text{kg}$ ) in rice calculated at different levels of damaged grains

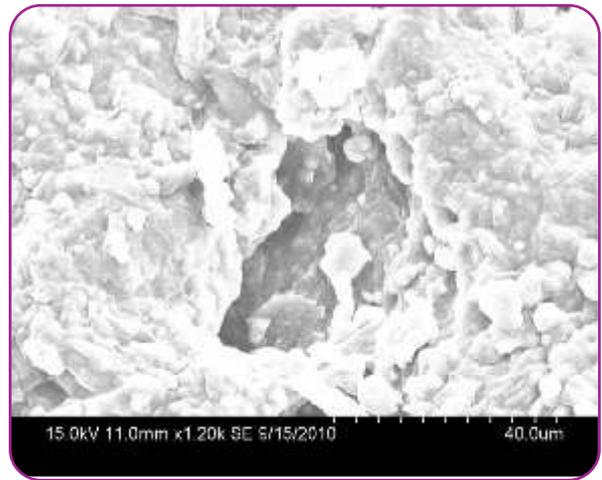
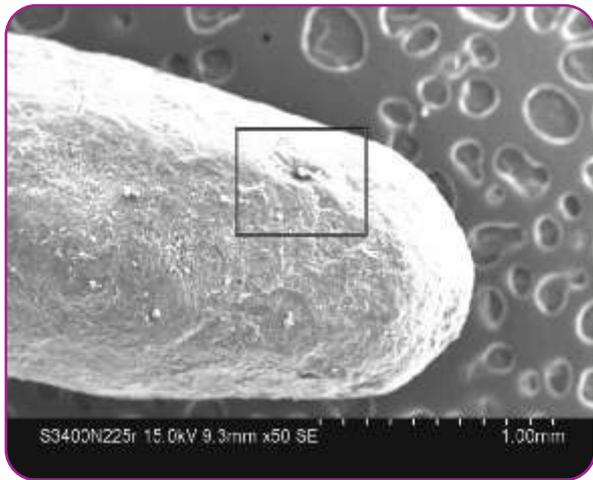
S.No.	Aflatoxin levels* ( $\mu\text{g}/\text{Kg}$ )	% Damage			
		3	4.75	10	12.3
1	74.7	2.24	3.54	7.47	9.22
2	7.72	0.23	0.37	0.77	0.95
3	151.3	4.54	7.17	15.13	18.68
4	10.7	0.32	0.51	1.07	1.32
5	77.7	2.33	3.68	7.77	9.59
6	74.5	2.24	3.53	7.45	9.20
7	36	1.08	1.71	3.60	4.44
8	64.4	1.93	3.05	6.44	7.95
9	16.4	0.49	0.78	1.64	2.02
10	73.6	2.21	3.49	7.36	9.09
11	BLOQ	-	-	-	-

\*data from 11 damaged rice samples

Table 50. Study Design for follow up analysis of PAU 201 rice samples

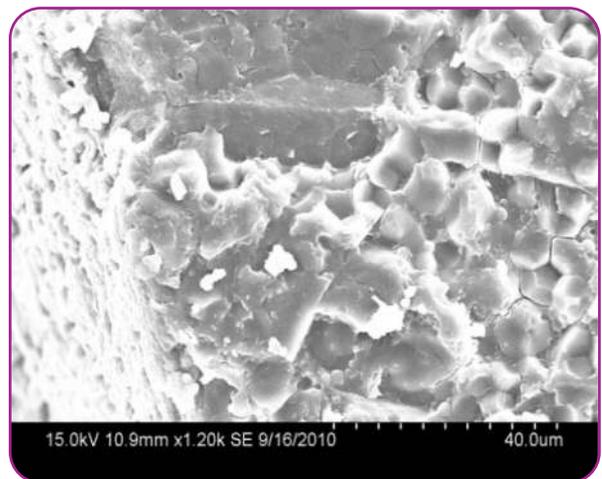
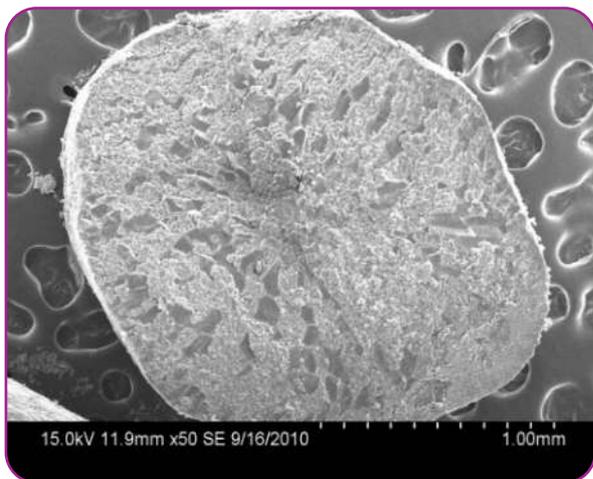
No	Sample	Processing	Sample for analysis	No.	Total	Expected result
1	PAU 201 paddy	Milling & polishing. Segregation of damaged grains	Damaged grains: Black tipped: Pin point damaged Other discoloured	3 categories (pooled from 35 samples) x 2 (duplicates)	6	Aflatoxin levels in damaged grains
2	PAU 201 paddy	Storage at RT. Milling & polishing every 6 months	Milled & polished rice at 6 months storage	35 x 2 time periods x 2 (duplicates)	140	Aflatoxin levels during storage
3	PAU 201 milled rice	i.Unpolished brown rice ii.Polished rice before sorting iii.Polished rice after sorting	i.Unpolished brown rice ii.Polished rice before sorting iii.Polished rice after sorting	11 x 3 types x 2 (duplicates)	66	Aflatoxin levels in rice at different stages of segregation
4	i.Local variety and ii.PAU 201 milled & polished rice	Spike local variety at 0,1, 5, 10, 20, 30, ppb level with aflatoxin	i.Local variety aflatoxin spiked sample ii.PAU 201 naturally contaminated with aflatoxin (total) iii.PAU201 w/o aflatoxin	i.6 spiked x 3 (triplicates) x 2 cleanup methods=36 ii.PAU201 naturally contaminated (3 levels) x 2 cleanup methods x 2(duplicates)=12 iii.PAU w/o aflatoxin 2 samples x 2 x2=8	56 (36+12+8)	Sensitivity of detection/quantification of aflatoxin using 2 cleanup procedures (conventional and immunoaffinity column)
5	i.PAU201 paddy ii.Damaged rice	Milling & polishing.	Milled & polished rice & damaged rice for estimation of ergosterol	35 x 2=70 11 x 2=22	92	Extent of fungal contamination based on level of ergosterol in rice sample.
Total					360	
Total No. of estimations: 360+ 10% error= 360+36=396						

**Fig 55. Scanning electron micrographs of pin point damaged PAU 201 rice grains**

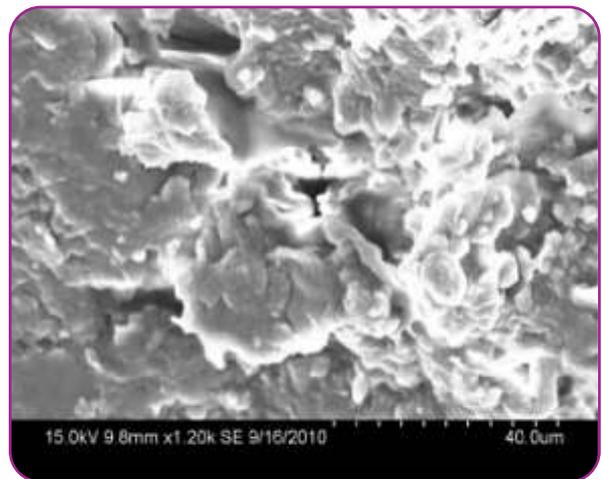
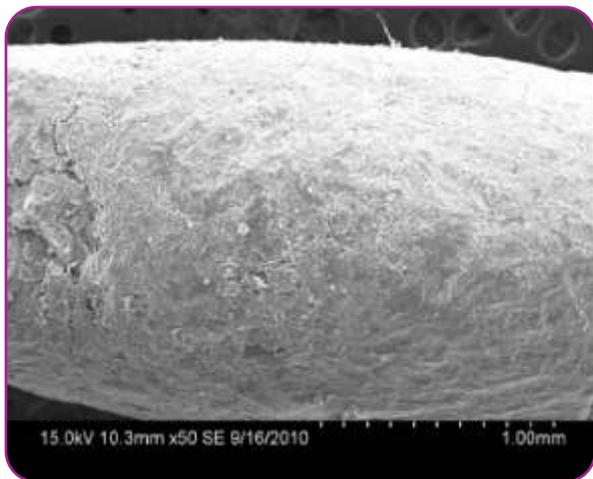


Surface sections at 50X & 1200X magnification showing affected areas (in box) and no fungal structures

**Fig 56. Scanning electron micrographs of partially damaged PAU 201 rice grains**

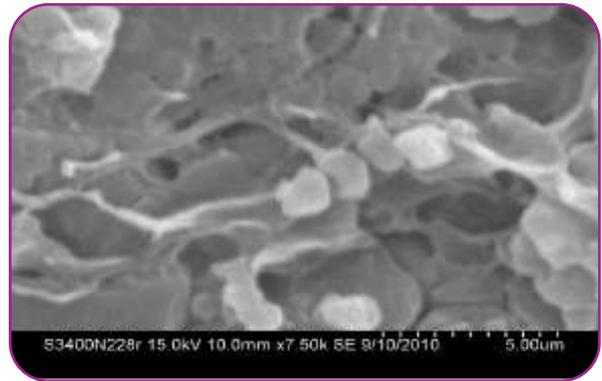
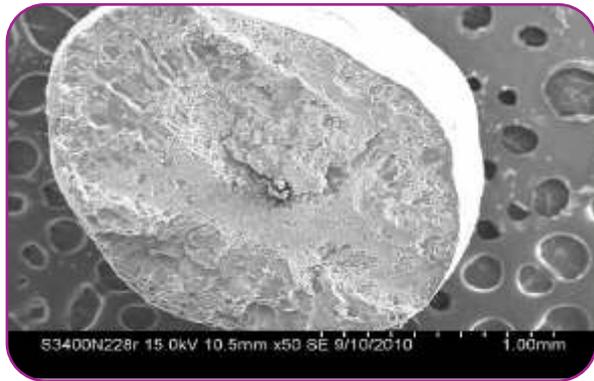


Transverse sections at 50X & 1200X magnifications showing normal structure

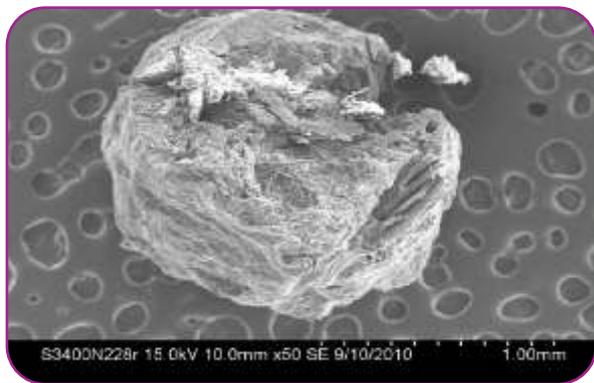


Surface sections at 50X & 1200X magnification showing affected areas (box) and no fungal structures

Fig 57. Scanning electron micrographs of fully damaged PAU 201 rice grains



Transverse section at 50X & 7500X magnifications showing apparently damaged areas (box) and fungal hyphae and spores (arrow)



Surface sections at 50X & 7500X magnifications showing irregular appearance & spore morphology

Fig 58. Prussian Blue staining of rice grains for detecting presence of iron



**AU 201 rice partially damaged**

Before staining



After staining



**PAU 201 rice completely damaged**

Before staining



After staining



## CONCLUSION

The results of the study indicated that the stored rice samples were in compliance with the food safety and standard regulations of the FSSAI with respect to aflatoxin contamination. The observations on fungal and aflatoxin contamination emphasize the importance of generating such data in damaged grains which in turn would aid in proper risk assessment for setting tolerance limits for damaged grains in food grains.

# IX. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

## A. SERVICE ACTIVITIES

### 1 Breeding and supply of animals

During the 12 months period, a total of 25996 animals were bred and out of which 22290 animals were supplied to various institutions (86%) including NIN for research. Compared to last year, the percentage of supply is almost equal. Proportionately, the income generated was Rs.52,54,470. The details of individual strains bred and supplied are shown in Tables 51 and 52.

### 2 Supply of animal Feed

#### a. Stock Animal feed

Total stock of 83,759 kgs feed (rat and mouse feed 70,649 kgs + guinea pig and rabbit feed – 13,110 kgs) was prepared during the period. Out of this, a total of 32,761 kgs feed (rat and mouse feed 26,381 kgs + guinea pig and rabbit feed 6,380 kgs) was supplied to outside institutions during the period generating an amount of Rs.34,95,735. An additional 50,998 kgs feed (rat and mouse feed 44,268 kgs + guinea pig and rabbit feed 6,730 kgs) was also supplied within the institute.

#### b. Experimental Animal Feed

In addition, the Centre prepared custom made experimental animal feed as last year and supplied 420 kgs this year and generated an income of Rs.3,61,682. The details of experimental feed supplied are given in Table 53.

### 3 Blood and Blood products

During this period, a total of 1339 ml of Plasma /Serum of blood have been supplied to 08 different Institutions on 21 different Occasions and an amount of Rs 1,71,230 has been collected.

### 4 Health Monitoring

During this period, a total of 736 samples were subjected to microbiological monitoring. Samples belong to different strains of Rats (WNIN 276, GR/Ob 230, and Ob/Ob 230). They were tested mainly for the specific bacteria like Celia Associated Respiratory Bacillus (CARB) Bacteria, Mouse Adeno virus, Pastruella pneumotropica, Helicobacter, Clostridium piliformis (CPIL, Hantan), by using ELISA method as the laboratory is under renovation. Samples were collected from different age groups in order to see their pattern of colonization. The results are furnished in the Tables 54-56.

In general, testing indicated that, WNIN rats showed the presence of organisms after the age of 6 months, where as in obese strain the organisms started appearing right from the first month onwards. When compared between the sex, females have more prevalence than males in both WNIN and in Obese animals. Among the organisms Helicobacter hepaticus, Pasteurella pneumotropica are more prevalent in both types of rats. MAd, CPIL and CARB were not present.

### 5 Genetic Monitoring

The Genetic monitoring facility functions to protect the genetic integrity of the inbred strains and transgenic lines of rodents utilized in the various research programs at NCLAS, NIN.

Table 51. Details of breeding and supply of different species and strains of laboratory animals during the period 1.4.2011 to 31.3.2012

S No	Species	Strain or Breed	Stock As on 1.4.11	Bred during The period	Total Number of animals							Balance as on 31.3.12
					Availa ble	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp. Old age	Sick	
1	Mouse	BALB/c An. N (inbred)	312	1589	1901	3	1265	1268	60	-	-	573
		C57BL/6J (inbred)	1112	1679	2791	101	1428	1529	556	64	-	642
		N:NIH(S) Nude (inbred)	215	290	505	3	171	174	172	-	-	159
		NCr.Nude	292	285	577	24	169	193	205	-	-	179
		FVB/N (in bred)	108	73	181	-	-	-	26	-	-	155
2	G. Pig	Swiss (in bred)	2343	6067	8410	304	6218	6522	335	-	-	1553
		N:HART (Hartley)	354	1094	1448	35	933	968	122	-	-	358
		N:NIH (Coloured)	214	563	777	-	478	478	83	-	-	216
3	Rabbit	New Zealand white	64	167	231	10	116	126	27	-	-	78
4	Monkey	Macaca mulatta (Rhesus)	24	NIL	24	6	-	6	-	-	-	18
TOTAL			5038	11807	16845	486	10778	11264	1586	64	-	3931

Percentage of animals supplied to other Institutions

**Table 52. Details of breeding and supply of different species and strains of laboratory animals during the period 1.4.2011 to 31.3.2012**

Sl. No	Species	Strain or Breed	Stock as on 1.4.11	Total Number of animals							Balance as on 31.3.12	
				Bred during The period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp. Old Age/ Sick		
1	Rat	CFY/NIN (inbred)	97	103	200	-	-	-	-	08	70	122
		Fischer 344 N (inbred)	120	483	603	80	19	99	02	80	422	
		Holtzman (inbred)	88	71	159	-	-	-	05	60	94	
		SD (Sprague Dawley) (Outbred)	876	4065	4941	794	3100	3894	-	150	897	
		Wkyoto (inbred)	62	80	142	3	-	3	13	60	66	
		WNIN (inbred)	2551	7187	9738	390	5580	5970	324	273	3171	
		WNIN/GR -Ob	926	455	1381	160	-	160	146	170	905	
		WNIN/Ob -Ob (inbred)	926	1020	1946	242	-	242	185	477	1042	
2	Hamster	Golden (inbred)	387	725	1112	-	658	658	43	-	411	
3	Sheep		1	-	1	-	-	-	-	-	1	
Total (Table 2)			6034	14189	20223	1669	9357	11026	726	1340	7131	
Table 1			5038	11807	16845	486	10778	11264	1586	64	3931	
Grand Total - Tables 1 + 2			11072	25996	37068	2155	20135	22290	2312	1404	11062	

Percentage of animals supplied to other Institutions

Rodents used in today's biomedical research must not only be free of disease that may influence experimental results, but also be well-defined in terms of their genetic makeup. This is especially true with the increasing use of transgenic mice in most areas of biomedical research. The genetic monitoring program has been designed to:

1. Construct genetic profiles for each strain/line maintained in the breeding colonies and provide routine genetic surveillance of the various strains for compliance to their profiles.
2. Provide for rigid enforcement of proper genetic management procedures within the breeding colonies. The genetic profile of a particular strain is composed of a set of genetic markers, which uniquely defines the strain and differentiates it from other inbred strains.

Researchers are increasingly aware of the importance of validating the genetic status of their research models. Accurate and efficient genotyping is critical for the validation and interpretation of the results.

Benefits of genotyping include more efficient breeding and faster production of research models. DNA based tests to facilitate the periodic genetic monitoring of laboratory rodents. Genotyping is most often performed by DNA amplification techniques.

**Table 53. Experimental feed supplied from 1.4.2011 to 31.3.2012**

S. No	To whom supplied	Type of diet	Quantity (Kgs)
1	NBRC, Haryana	Iron Def. Diet	15
2	CCMB	Maltodextrine	20
		High fat	16
3	St. Johns, Bangalore,	Iron def	25
4	Leila Nutraceuticals, Vijayawada	Fructose	4
5	IGIB, Delhi	Fructose	47
		High fat	35
6	Annamalai University, Tamil Nadu	High fat	15
7	Hamdard University, Delhi	High fat	55
8	Vimta	Maltodextrine	15
9	S.V.University, Tirupati	High fat	49
10	SCTIMSCT	Def.	50
11	S.K.University, Ananthapur	Fructose	33
12	SCTIMSCT	Def.	25
13	Jiwagi Unviersity, Gwalior	8% Protein 20%	10
14	CVsC, Rajendra Nagar	High fat	20
15	GNTC, Hyd	Lithogenic	1
16	S.K.University	Fructose	4
17	NRI (Ayurveda), Gwalior	High fat	20
TOTAL			420

**Table 54. Results of testing of Rat of WNIN**

S. No	Age in months	1	2	3	4	5	6	7	8	9	10	12	13	Results
	Number & Sex ratio	F-M	F-M	F-M	F-M	F-M	F-M	F-M	F-M	F-M	F-M	F-M	F-M	
	Tested Virus / Bacteria	----												
1	Helicobacter	----	1+1	0+0	0+0	0+0	2+1	2+1	2+0	2+2	2+0	1+2	1+0	13/24: 7/22
2	Pasteurella pneumotropica	----	0+0	0+0	0+1	0+0	0+1	0+0	1+0	0+0	0+0	1+0	1+0	3/ 24: 2/22
3	MAd	----	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0/24: 0/22
4	CARB	----	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+1	0+0	0/24: 1/22
5	CPIL	----	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0/24: 0/22
6	Hantan	----	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+1	0/24: 1/22

**Table 55. Results of testing of Ob/Ob GR/Ob Rats**

S. No	Age in months	1	2	3	4	5	6	7	8	9	10	12	13	Results	
	Number & Sex ratio	F-M 1+1	F-M 4+4	F-M 1+2	F-M 4+8	F-M 6+2	F-M 11+10	F-M 4+6	F-M 1+1	F-M 2+2	F-M 7+3	F-M 2+0	F-M 2+8		F-M 45+47
	Tested Virus / Bacteria														
1	Helicobacter hepaticus	0+0	1+2	0+0	0+1	1+1	2+2	----- -	0+1	-----	6+1	0+0	0+3	10/45: 11/47	
2	Pasteurella pneumotropica	1+0	1+0	0+0	0+2	1+0	2+1		1+1	-----	4+1	1+0	0+5	11/45: 10/47	
3	MAd	-----	0+0	-----	0+0	0+0	0+0	0+0	----- -	0+0	0+0	-----	0+0	0/45: 0/47	
4	CARB	-----	0+0		0+0	0+0	0+0	0+0	----- -	1+0	1+0	-----	0+0	2/45: 0/47	
5	CPIL	-----	-----	-----	----- -	-----	-----	----- -	----- -	-----	-----	-----	-----	-----	
6	Hantan	-----	0+0	-----	0+0	0+0	0+0	0+0	----- -	0+0	0+0	-----	0+0	0/45:0/47	

About 11 different loci located on different chromosomes of rat were monitored. The microsatellite analysis of rat strains was done utilizing PCR techniques. PCR methodology used to monitor the strain specific marker. More primers were designed to genotype the rat strains maintained at NCLAS to establish the genetic monitoring facility at NIN. The genotyping work to develop DNA markers linked to strain specificity had been initiated.

Genomic DNA isolated from tail tissues (N=6) of SD, Fisher 344, CFY, WKY, WNIN, WNIN Ob/Ob (Obese, Carrier and lean) WNIN GR-Ob/Ob (Obese carrier and Lean) strains. Genomic DNA subjected for genotyping with SSLP primers using PCR for amplification and genotyping. Among the 11 primers three primers (R117, R 148, R 196) are showing differences among the strains. These three primers were linked to rat genome i.e. D6WOX10 and D4 WOX 18.

**Table 56. Summary of testing & Results of Microbiological Monitoring in WNIN and Ob/Ob rats**

S No	Virus/bacteria	No. of samples Tested & Result	
		WNIN rats	Obese rats
1	CARB	1/46	2/92
2	Mouse Adeno Virus	0/46	0/92
3	Pastruella Pneumotropica	5/46	21/92
4	Helico bacter	20/46	21/92
5	CPIL	0/46	-----
6	Virus	1/46	0/92
	Total	27/276	44/460

When analyzed on agarose gel (2.0%) resolution of PCR products were not clear. Further, analysed, amplified PCR product using poly acrylamide gel to get a better resolution. The polymorphism was identified among the strains genotyped. Linkage of polymorphic alleles to specific strain need to be performed which requires genotyping of large number of animals within the rat strain.

## 6 Human Resource Development

In the junior level training course (LATTC), during this year, there were 10 participants trained and in the senior level supervisory training course (LASTC) level there were 11 participants. Ad-hoc training was given to 4 candidates for a period varying from one week to 4 weeks.

The Centre celebrated World Laboratory Animal Day on 24<sup>th</sup> April 2011 and conducted a symposium to discuss about the newly introduced Animal Welfare Act 2011 in replacement to the existing PCA Act 1960 by the Ministry of Environment and Forests Govt. of India. About 250 members have participated from various institutions in the panel discussion and resolutions were passed to forward it to the Ministry through Indian National Science Academy. During the celebrations, well known laboratory animal personnel were also felicitated.

The centre also conducted a CPCSEA symposium on Role of IAEC members in the approval and conduct of animal experimentation on 20<sup>th</sup> May 2011. There were about 40 participants from different institutions from within AP.

In addition, the center organized Indo Australia Obesity Institute - Scope Workshop on 3<sup>rd</sup> and 4<sup>th</sup> June, 2011 to discuss various aspects of collaboration projects of common interest mainly concerning obesity between the two countries to establish an Indo-Australia Obesity Research Centre at NIN at a later date. Participants consisted of scientific personnel from NIN and other leading CSIR and ICMR Institutes working on obesity in India and 10 leading scientists working in Australian Research Institution.

## 7 Research Support

40 research projects were evaluated and approved by IAEC for animal experimentation during the period. These projects were from NIN and Pre-Clinical Toxicology (PCT) research group.

## B. RESEARCH ACTIVITIES

### 1 Establishment of baseline values of body composition and blood pressure in different species of laboratory animals maintained at NCLAS, NIN

National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN) has been providing different species and strains of laboratory animals for in-house use as well as to outside supply and also to preclinical toxicology testing. As a part of the activity of the centre, it was decided to establish the normal values of the animals maintained at the facility. The determination base line values like body composition, locomotor activity, blood pressure and clinical chemistry parameters in rat strains maintained at the centre were completed. The base line values of Guinea pigs (g.pigs) and hamster in-terms of their body composition and clinical chemistry parameters have measured. The study was subsequently extended to New Zealand white rabbit strain maintained at the centre. During the year 2010-11, the study with an aim to establish the body composition [lean body mass, fat, fat%, by TOBEC, bone mineral density (BMD) and bone mineral concentration (BMC) by DXA] of rabbits of both sexes were initiated.

#### *Work done during the year*

12 males and 12 female's NZW rabbits with body weight ranging between 3.5 to 4.0 Kg were taken and their body composition, hemogram and clinical chemistry parameters were determined. Preliminary data showed that DXA was a better method to determine the regional wise fat distribution, BMD and BMC and TOBEC for lean body mass (LBM) and extra cellular fluids in rabbits.

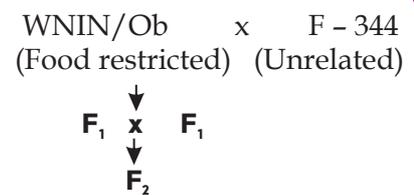
LBM, BMC and BMD levels were found to be high in males compared to females. However, total body fat and fat % levels were found to be high in female rabbits compared to males. The fat depositions were high in lower portion of the abdomen (retroperitoneal region) of females compare to males. The hemogram analysis of rabbits revealed that males showed higher values than females. However, the haemoglobin levels were high in females compared to males. In clinical chemistry of rabbits, glucose levels are significantly higher in females compared to males. However, these levels were agreeing with the literature cited values. No significant difference was seen in total protein, albumin, calcium, creatinine and SGOT enzyme levels in both genders of rabbits. Female rabbits had higher levels of urea, alkaline phosphatase enzyme compared to males. The overall picture of the haematology and clinical chemistry in rabbits very much correlated with the literature cited values. Statistical analysis of the data is in progress.

## 2 Localization and cloning of obesity gene in wnin mutant rat (WNIN/Ob)

WNIN/Ob is a mutant obese rat strain, isolated and established from the parental rat stock of WNIN. Physical and biochemical characterization of these animals have been done earlier and it was shown at molecular level that leptin and its receptor genes (mutation of which causes obesity in other known rodents models) are unaltered in these mutants. The present project envisages identifying and localizing the gene responsible for obesity in WNIN/Ob rat through gene mapping and positional cloning.

### Work plan

WNIN/Ob mutant rats were crossed with Fisher - 344, an unrelated strain and genetic analysis were carried out for parents and F2 progeny, to understand the exact locus mutation.



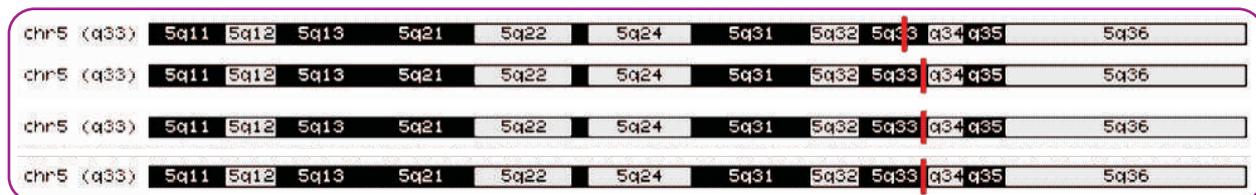
### AIMS AND OBJECTIVES

To identify the gene responsible for obesity in obese mutant rat (WNIN/Ob).

### Work done during the year

The parents, [5 male (WNIN(Ob/Ob), 13 female (Fisher-344)] along with 80 F2 obese and 6 WNIN rat genomic DNA were genotyped with 95 SSLP markers using ABI 3730 genotyper. The data was analyzed and a polymorphic region on chromosome number 5 was identified. RGD (Rat data base) and UCSC genome information sites were browsed for the selection of more 40 SSLP markers on the basis of their polymorphic history and proximity to the markers showing polymorphism. The 80 mutant animals were genotyped with about 40 SSLP markers. Among the markers used, 8 were showing polymorphism and this spanned an area of about 1.3 mega base genomic region. This genomic region is considered as the hot spot for the of mutation responsible for obesity in WNIN obese rats.

Polymorphic region on Chromosome No. 5 (Hot spot) (Fig 59)



**Target genomic regions sequencing**

For the target sequencing, a region length 3.27Mb as mentioned above were considered after masking repeats and leaving gaps of 2.23 Mb (Repeat and Gap length 1.04Mb). This area covering both sides of the 'hotspot' was subjected for sequencing (Fig 59), using high throughput genotyping technology ie on Illumina genotyping platform. Co-ordinates of this region were selected as per marker location obtained from RGD (Rat Genome Database) covering about 13 genes and the genome sequence of these were retrieved from UCSC genome browser. Probes were then designed for targeted region using Agilent e-Array. Genomic DNA was isolated from WNIN, Lean and Obese rat tails after shearing it by sonication, to make 800bp. These were then subjected to sequencing using Illumina (Illumina GAIIX at Genotypic Technology Pvt.Ltd. Bangalore) protocols of PCR amplification and Hybridization. Comparative analysis was carried out among the three along with reference sequence to understand the polymorphism in the sequenced genomic region (Fig 60). The analysis conducted gene wise was to know the number of deletions, insertions and SNP i.e. heterozygous and homozygous compared between the Wistar sequences in comparison with reference sequence (Table 57 & 58, Fig 61 & 62).

**Fig 60. Sequenced chromosome position marked**



When the SNP was compared among the WNIN strains, numbers of deletions were found to be more between the WNIN and WNIN/Ob, than with that of WNIN/Ob lean; while the deletions were less between WNIN/Ob and WNIN/Ob lean. However, more of a single SNP was noticed to be common among all the WNIN rats sequenced, when compared with reference sequence (Fig 61).

**Table 57**

S.No	WNIN	WNIN/Ob	WNIN/Ob-lean
No. Insertions	443	425	387
Deletions	447	416	470
<b>SNP</b>	3409	3156	3126
Heterozygous	1333	1059	1007
Homozygous	2076	2097	2119

**Fig 61. Comparative analysis of sequenced genomic region between WNIN strain and with reference sequence (Brown Norway)**

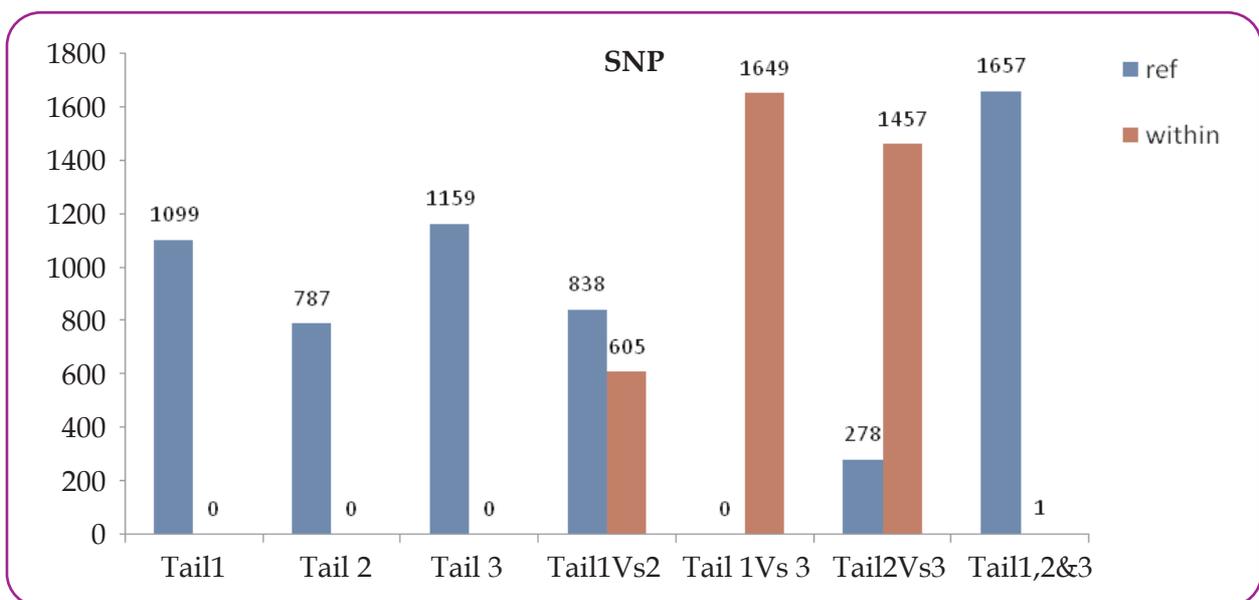


Fig 62. Gene wise SNP analysis

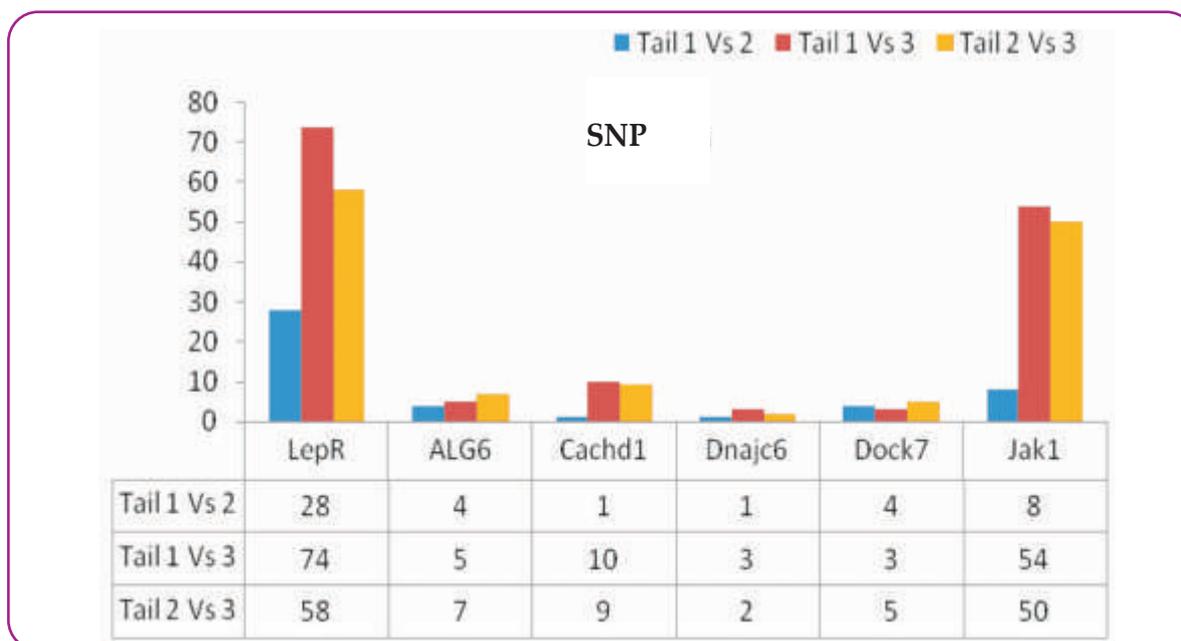


Table 58. Gene wise deletions analysis

	LepR	AK4	ALG6	Angptl3	Adg4	Cachd1	Dnajc6	Doc k7	Efca b7	Itgb 3bp	Jak1	Usp1	Pgm1	Raver	Ror1
Intron	21	14	1	1	12	10	4	1	3	6	7	0	13	20	95
Exon	6	0	0	0	0	0	1	0	1	1	3	2	0	0	0
5'UTR	25	0	0	0	0	0	0	0	0	0	14	1	0	0	0
3'UTR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

In general, high number of deletions was noticed in intron region than in exon. It was very interesting to see that the deletions were absent in 3' UTR of all the genes studied, while more deletions were seen in 5' UTR region, especially in LepR and JAK1 genes, in that order with a single deletion in Usp1 gene. The SNP were further validated to understand their location on genome and its variation with position to reference sequence. It was surprising to notice that each strain has its specific SNP located on the chromosome 5.

The identified sequence positioned in LepR gene was validated with known/ reported SNP using bio-informatics tools and through this approach, the WNIN/Ob rat specific mutation was identified. The located coding sequence of 2679bp was found to be unique, which is a heterozygous SNP with zero degeneracy. Due to change in the A/R the coding amino acid is changed from acidic to base. It was noticed that a specific SNP in WNIN/Ob lean Lepr gene positioned in an intron G/S had changed and this also was heterozygous. Further in the WNIN rat, a mutation was observed, which is specific to ancestral strain and the SNP change is G/V located in a coding sequence (2679bp). Hence, it can be conclusively said that these rat phenotypes have specific mutations and are not correlated. In summary, the relation between the genomic mutation and its relation with exon change and alteration in the associated amino acid had identified .

The restriction sites with in the above SNP site were analyzed using bio-informatics tools and appropriate primers were designed to probe the genome of WNIN/Ob and its crossed F2 population with F-344, to understand its role through quantitative analysis of transcripts

generated. The work is in progress and hoping that such an analysis will confirm the mutation suspected to be responsible for the development of obesity in the WNIN/Ab rats.

#### **Future Direction and course of action**

##### ***Gene expression and Exon trapping***

Primers could be designed for quantitative PCR to compare the level of expression of the RNAs in mutant and ancestral WNINrats. If differential expression is noticed it might be considered additional methods for gene identification such as exon trapping to address the possibility that the WNIN obesity gene has not been annotated in the available databases. This could be further validated by Northern blots using RNA prepared from a panel of tissues isolated from mutant WNIN Ab/Ab and WNIN rats to screen for differences in mRNA size or expression level in ancestral Vs mutant rats.

##### **Confirmation of Gene Identity**

It could be confirmed that the above mutant locus as a candidate gene for obesity by creating an ES cell derived mouse with a knockout in that gene. Pending the outcome of these confirmatory studies, it could also make recombinant protein to probe its biochemical activities, make antibodies directed against it and further study its regulation and function.

## X. PRE-CLINICAL TOXICOLOGY RESEARCH CENTRE

### 1 Assessment of allergenicity potential of novel proteins expressed in genetically modified (GM) plants under varying conditions of digestion and thermal treatments

Bejo Sheetal Pvt. Ltd. acquired Bt brinjal (event 142) with Cry1Fa1 gene from Indian Agriculture Research Institute (IARI), New Delhi in 2005. Then back crossing and direct hybrid development activities were started. In 2008-09, green house evaluation was completed and in 2008-09 and 2009-10 BRL- I trial in open field was conducted, and an intention to promote it as an insect resistant crop. The investigation was undertaken to assess the allergenicity of Cry1Fa1 recombinant protein by *in vitro* pepsin digestibility assay in Simulated Gastric Fluid (SGF) as per the protocols and guidelines issued by Department of Biotechnology (DBT,2008) and Indian Council of Medical Research (ICMR, 2008), Government of India. Bejo Sheetal LTD have developed Bt brinjal containing Cry1Fa1 gene with an intention to promote it as a pest free crop.

#### METHODOLOGY

**I. Pepsin digestibility assay:** This was performed on Cry1Fa1 recombinant protein using protocol standardized earlier in the project. The following investigations were performed as part of the assay:

- a) *Characterization of test protein by SDS-PAGE:* The recombinant test proteins provided by sponsor was HPLC purified protein with >70% purity in solubilized in sodium carbonate buffer (50mM, pH 10.0). The test protein was characterized by SDS-PAGE with BSA as reference protein.
- b) *Estimation of test protein conc. by BCA assay:* The conc. of Cry1Fa1 recombinant protein was assessed by BCA assay. BSA at a conc. of 1mg/ml was used as reference protein.
- c) *Determination of limit of detection of test proteins in SGF by SDS-PAGE:* A limit of determination assay was performed prior to the digestion assay to ensure the minimum level of protein that can be detected so as to detect 90% digestibility of test proteins. Serially diluted test protein samples in SGF at pH1.2 representing 2.5-200% of target protein conc.were loaded on 10-20% Tris glycine gels. After separation of test protein samples gels were stained and analysed by densitometry.
- d) *Determination of pepsin digestibility of test proteins:* The digestion of Cry1Fa1 recombinant protein was performed in SGF at pH1.2. The pepsin to test protein ratio was maintained at 10units of pepsin activity to 1µg of protein. The test protein was added at a conc. of 170µg/ml SGF containing 1700 Units of pepsin activity. Pepsin activity was verified prior to digestion reaction by estimating the amount of soluble peptides present in trichloroacetic acid solution after pepsin digestion of denatured haemoglobin. Pepsin activity determined from three replicates showed a specific activity of 3337U/mg solid that was within the acceptable range of 1000 units of the activity reported by manufacturer. The digestion assay was carried out at 37C in a water bath. Aliquots of pepsin reacted protein were drawn at various time periods ranging from 0 to 60mins minutes of incubation were drawn, neutralized in carbonate buffer (pH11) and subjected to SDS-PAGE analysis. Analysis of separated bands on SDS-PAGE gels were carried out by densitometry using Kodak image

software. The stability of the protein was assessed as the time required to achieve 90% digestion between 0.5-60 minutes which was estimated based on the shortest time-digested sample with band intensity equal to or less than the 10% of the undigested protein.

## RESULTS

### *Characterization of test proteins:*

The recombinant protein solubilized in sodium carbonate buffer (50mM) at pH 10 and HPLC purified showed a molecular weight of 70kDa on SDS-PAGE (10-20% Tris glycine) (Fig 63) and a conc. of 2mg/ml as determined by BCA assay.

### *Limit of detection of test protein:*

The stained gel consisting of series of test protein concentrations demonstrated a clear pattern of reduced intensity of stained bands with decreasing concentration (Fig 64.). The minimum amount of Cry1Fa1 recombinant protein that could be detected was 0.025g which is 2.5% of target protein conc. of 1.0g/well respectively (Fig 64).

Specific net intensity values from the image system (Kodak Gel Logic 100 ) plotted as regression line showed good fit ( $r^2=0.95$ ) (Fig 64.). The level of sensitivity observed for Cry1Fa1 recombinant protein was found to be sufficient to detect 10% residual protein in the digest.

***Pepsin digestion of Cry1Fa1:*** The results of the pepsin digestibility assay showed that the recombinant protein was rapidly degraded by pepsin in SGF at pH1.2 and 90% digestibility was achieved within <0.5mins (Fig 65). No stained bands were visible beyond the digestion control at time 0. No stainable bands that had intensity equal to or less than the 10% undigested test protein control (P1/10 Lane 12, Fig 65) were observed. Further analysis of the digested test protein at short time intervals spanning 0 to 5 minutes showed that the test protein was rapidly degraded within 15 secs of digestion in SGF at pH1.2.(Fig 66).

### *ii. Assessment of thermal stability of Cry1Fa1 recombinant protein:*

The recombinant test protein was diluted to a conc. of 1mg/ml buffer. A total volume of 100µl with a conc of 1µg/µl was subjected to heat treatments at different temperatures. The test sample controls consisted of untreated test protein samples at 1µg/µl conc. and 10% of the target conc. (0.1µg/µl). Separate samples were incubated at 25, 37, 55, 75 and 95°C for 30 minutes before cooling on ice. All the treatments were carried out in duplicates. Biological activity of the heat treated test proteins was performed in collaboration with sponsor Bejo Sheetal Seeds Ltd. Jalna at their facility. Biological activity of the proteins was tested in an insect bioassay using target insect namely, *Leucinodes orbonalis*. The heat treated test proteins were prepared at a conc. of 0.54µg/ml diet for a total volume of 10ml diet. The assay was performed by releasing first instar larva into the diet (10 per treatment) at RT. The mortality rate was recorded for every 24, 24, 72 and 96h and the final reading taken on completion at 96h. The heat treated test protein samples were also analysed by SDS-PAGE on 10-20% Tris Glycine gels along with untreated control test proteins.

Fig 63. Characterization of Cry1Fa1 recombinant protein

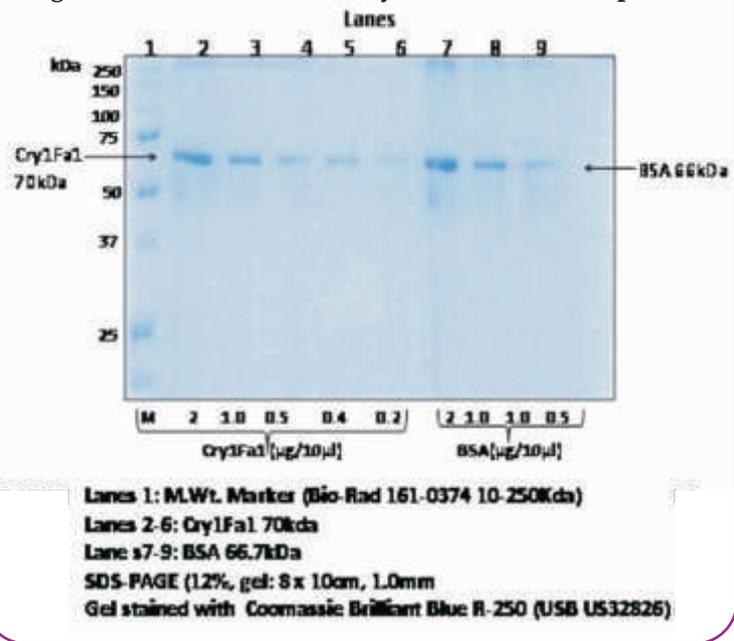


Fig 64. Limit of determination of Cry1Fa1 recombinant protein

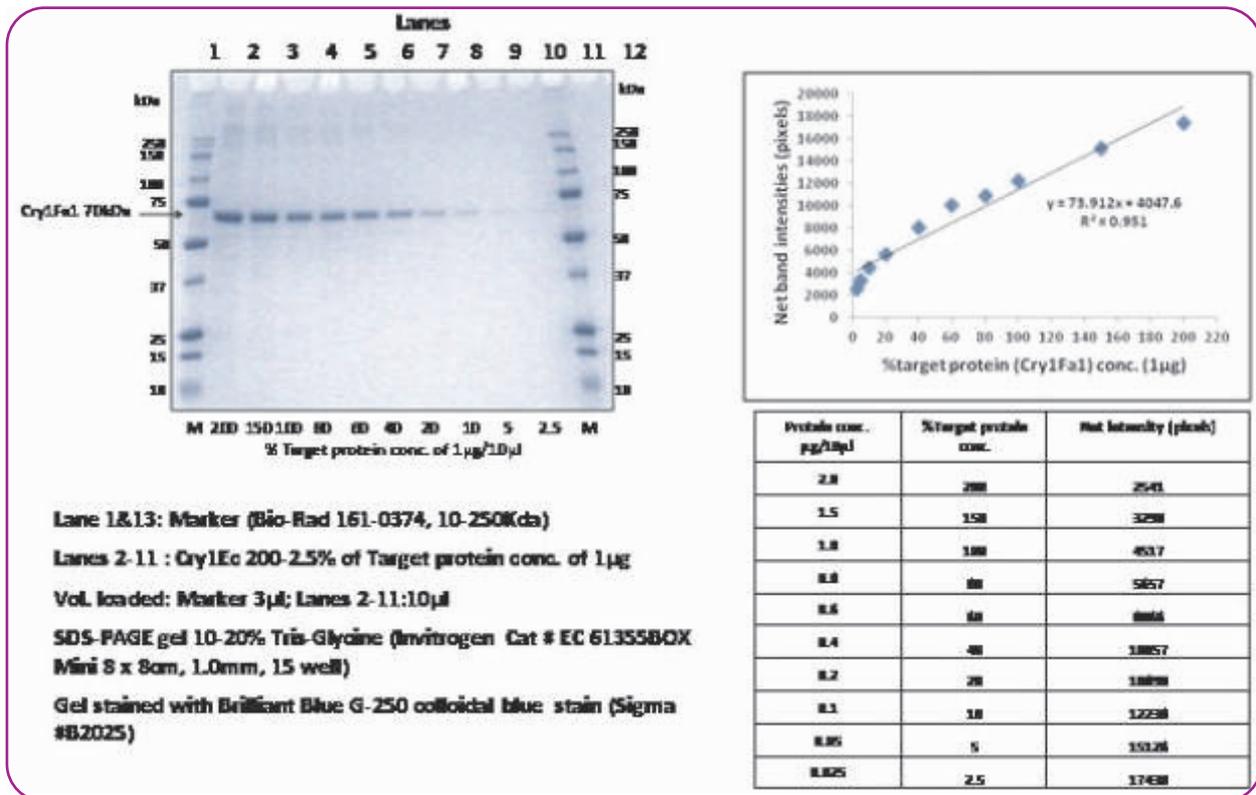


Fig 65. Pepsin digestion of Cry1Fa1 recombinant protein in SGF pH1.2

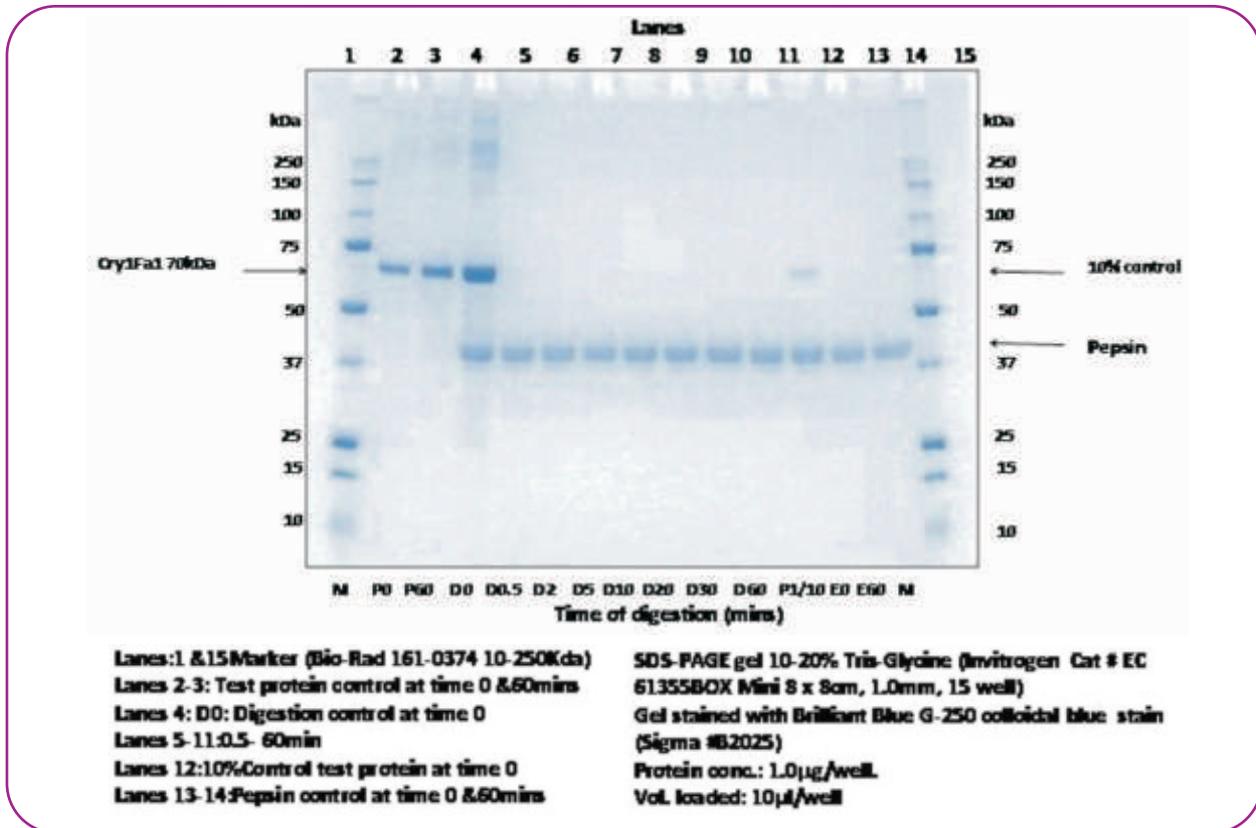
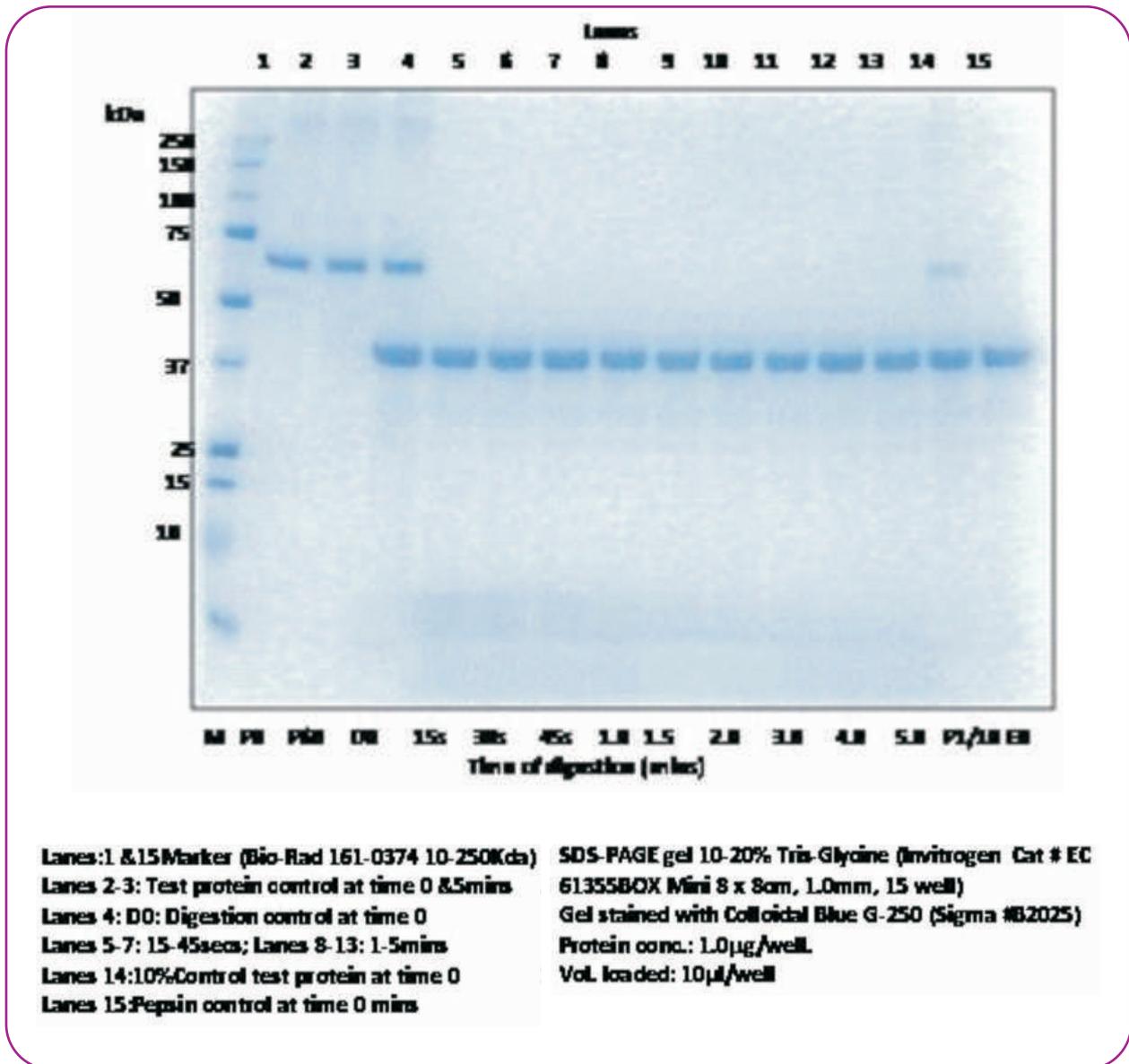


Fig 66. Pepsin digestion of Cry1Fa1 recombinant protein in SGF pH1.2 (0-5min)

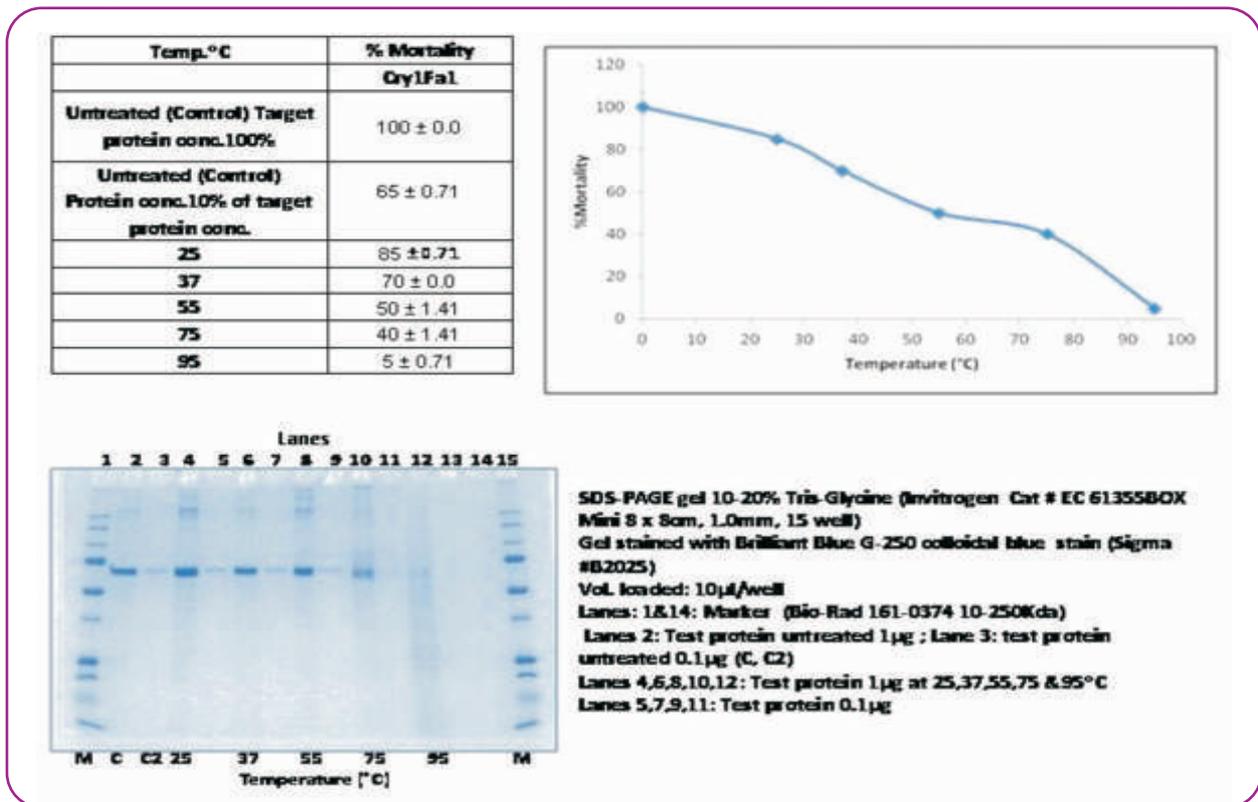


The stability of the recombinant proteins at a defined temperature was determined from the biological activity remaining after 30 minute incubation at that temperature. Proteins with more than 50% biological activity remaining were considered stable at that temperature. Proteins with between 50 and 10% biological activity were considered partially stable and proteins showing less than 10% biological activity were considered labile at the relevant temperature (DBT 2008).

## RESULTS

Insect bioassay of heat treated Cry1Fa1 recombinant protein showed that at a temperature of 95°C the mortality of target insects fed was zero indicating heat liability of the recombinant protein at this temperature (Fig 67). The mortality at 10% target protein conc. was 65%. SDS-PAGE analysis showed that the band intensity of protein sample heated at 95°C was less than the untreated control at 10% conc. (Fig 67).

Fig 67. Thermal stability assay of Cry1Fa1 recombinant protein and insect bioassay



## OUTCOME

The report on the results of the pepsin digestion and thermal stability assays of Cry1Fa1 recombinant protein have been submitted to regulatory agency for premarket approval.

## 2 Sub-chronic toxicity study of fruit and leaf of Bt okra containing Cry1Ac gene in WNIN rats

Genetic modification using recombinant DNA technology has led to the development of new agriculture plants with better quality. The attempts were on to develop Bt Okra. Despite the benefits and economic advantages of such crops, bio-safety issues of such products need to be evaluated. The DBT has recently updated the guidelines for evaluating the pre-clinical safety profile of Bt containing products.

Mahyco has developed Bt okra containing Cry1Ac gene for insect tolerant trait. Therefore, the present investigation was undertaken to evaluate Pre-clinical Safety Evaluation in WNIN rats by feeding orally daily for 90 days as per regulatory guidelines of DBT.

### METHODOLOGY

Mahyco, an agri biotechnology company has established the pre-clinical safety of pure/equivalent protein Cry1Ac in acute toxicity test using mice. This Cry1Ac protein had been incorporated to produce Bt okra. Therefore, sub-chronic toxicity test was carried out by feeding Bt okra and non- Bt okra daily for 90 days to rats as per regulatory guidelines of DBT.

The Sub chronic investigation was conducted in WNIN rats 60 (30M+30F), which were divided randomly into three groups viz., control, non transgenic [NTMDI] and transgenic [T(MDI)]. All animals were acclimatized and were trained for 10 days to eat the lyophilized okra fruit powder containing transgenic and non-transgenic material equivalent to MDI followed by regular diet. The control group received regular diet whereas, lyophilized non-transgenic (Local cultivator) and transgenic (Bt) okra was received by NT (MDI) and T (MDI) groups respectively. The rats were monitored bi-weekly for live phase, cage side, physical and neurological parameters. The hematology, clinical chemistry profile, gross necropsy and histopathological observations of all organs along with serum IgM, IgA and IgE levels were estimated at 48 hrs (92<sup>nd</sup> day) and 15<sup>th</sup> (107<sup>th</sup> day) day of post exposure to Bt and non-Bt diet.

## RESULTS

There was no mortality in any group of animals which were fed with non-transgenic or transgenic test material till end of the experimental period. Significant reduction in body weight gain was observed in both non-transgenic and transgenic groups as compared to control. Between the transgenic and non-transgenic no significant difference was observed. No abnormal changes in clinical chemistry profile was observed in any group except significant reduction in total proteins in non-transgenic groups. No changes in live phase, physical activity and neurological activity between the control and test groups were recorded throughout the study period. Humoral immunity as assessed by total IgM, IgA and IgE were within normal range in Bt and Non Bt rats. Though, there were statistically significant changes in hematological parameters, they were in normal range and hence not considered significant. There were no significant gross necropsy observations while the various histopathological changes are common to colony bred animals and also seen mostly across all groups and hence not considered significant.

3

There was no mortality in any group of animal which received transgenic or non-transgenic material till the end of the experiment. The animals fed with transgenic and non-transgenic test material did not show any significant changes in body weight. Serum Immunoglobulin levels (IgM, IgA and IgE) were not altered with Bt or non-Bt. Clinical chemistry profile was also normal in all groups of animals exposed to transgenic and non-transgenic Okra. In spite of significant alteration in RBC, Hb all were in normal range. These were gross necropsy and histopathological changes recorded which can be attributed due to test material exposure. Hematological and histopathological parameters studied were found to be non significant and cannot be attributed to the chronic (90 days) exposure of Bt okra.

3

## Pre-clinical toxicity evaluation of recombinant granulocyte-colony stimulating factor (GCSF)

G-CSF is naturally produced special proteins in the body, a haematopoietic growth factor which promotes white blood cells in chemotherapy. They can also be made as a drug. Shasun Chemicals and Drugs Limited have recently developed GCSF using recombinant DNA technology with an intention to promote it for treatment of cancer. So it becomes mandatory to undertake its safety evaluation as per the guidelines of DBT and Schedule Y of DCGI for recombinant products.

## METHODOLOGY

The safety of test compound has been established by acute toxicity test in mice and rats by IV and IM route of single ten times of intended therapeutic dose. Since it is classified as bio-similar the 28 days sub-chronic toxicity test in Swiss Albino mice and New Zealand White rabbits at three dose levels viz., Therapeutic dose (TD), Average dose (AD, five times of TD) and High dose (HD, ten times of TD) has been conducted by IV and IM route. The observation for live phase; cage side observations, physical examination and neurological activities along with clinical chemistry, hematology and histopathology of all major organs were monitored. In addition, blood was collected on 14<sup>th</sup> day of exposure for the estimation of Absolute Neutrophil Count (ANC).

## RESULTS

**The Acute toxicity test:** No mortality was observed to test material administered by subcutaneous route in 10 times of intended therapeutic dose. All the animals were found to be active and no significant changes in body weight and food intake were recorded.

In sub-chronic toxicity study, mortality of two male mice (2<sup>nd</sup> day and 11<sup>th</sup> day) in therapeutic dose group and high dose group respectively that received the test compound by I.V. whereas lethality in S.C one male mice (8<sup>th</sup> day) and one female animal (12<sup>th</sup> day) in vehicle control and innovator group respectively was observed. No mortality was noted in rabbits which received test compound by IV/ IM route.

In all survived animals, no major abnormal clinical signs were observed in other animals during and after post exposure to test compound administration. There were no differences in body weight gains, live phase, physical activity and neurological activity between the control and test groups throughout the study period. The clinical chemistry parameters viz., blood glucose levels, kidney and liver function tests were found to be in normal range in all groups of animals exposed to the test compound when compared to vehicle control. ANC counts at the end of 14<sup>th</sup> day were elevated only in HD group, while WBC were raised in mid term only and not at final term.

## CONCLUSIONS

- In acute toxicity test, no mortality was recorded in mice after single exposure of 10XTD by SC route.
- In sub-chronic toxicity, mortality (5%) was recorded but it was not observed in rabbits. No abnormalities in body weight gains, live phase, physical activity, biochemical profile. Histopathology evaluation showed changes in experimental groups which were seen in VC group also and hence not considered significant.

4

## Pre-clinical safety evaluation of red gram pulses with ferric ammonium citrate

Iron is an essential mineral required for growth, development and maintenance of physiological systems. Iron deficiency anemia has been a public health problem particularly in populations subsisting on predominantly vegetarian diets. Commonly consumed cereal-based foods are low in iron and high in tannins and phytates which irreversibly chelate dietary iron and thereby limit the absorption of dietary iron. Therefore, poor density and bioavailability of dietary iron is the major etiological factor for the widespread prevalence of iron deficiency anemia in the country, particularly in children and pregnant women. The protein (particularly animal protein) and ascorbic acid are known to enhance the absorption of iron in humans and can overcome the inhibitory effects of phytic acid<sup>2</sup>.

Food fortification is one of the important sustainable suggested by WHO. In view of common usage of Red gram (*tur dhal*) and to provide bio-available iron, VASMO Food Co. (New no. 336, (O). 166, Thambu chetty street, 3rd floor, Chennai - 600 001, India.) has developed a tur dhal fortified with ferric ammonium citrate (0.175%) and ascorbic acid (0.004%) using indigenous technique for human use.

The investigation was undertaken to assess the pre-clinical safety profile of red gram pulses fortified with ferric ammonium citrate as per Food Adulteration Act - 1954/ Schedule y of DCGI.

The investigations involved acute and sub-chronic toxicity test of fortified *tur dhal* given to mice/ rats in various intended daily dietary intake (DDI) levels. The ratio of intended DDI was calculated based on NNMB data on pulses consumed by human population.

The acute toxicity tests (14days) in *Swiss Albino* mice and *Sprague Dawley* rats with single exposure of 10 and 20 - X of DDI were conducted. This was followed by sub-chronic toxicity test (28 days) in 4-6 weeks old SD rats (54 Females + 54 Males) divided equally in five groups viz. (i) Normal control (ii) Vehicle control (iii) 1X DDI (iv) 2.5 X DDI and (v) 1X DDI-PD. The Normal groups have received NIN standard diet, whereas Vehicle control has received *tur dhal* in pellet form, while 1XDDI and 2.5XDDI groups have received 1X and 2.5 X Iron fortified *tur dhal* respectively, daily for 28 days in addition to the NIN standard diet as per SOP (Appendix-VII). The 1X DDI-PD group has received 1X- fortified *tur dhal* in animals which were restricted for protein (14%) (PD), so that it mimics poor man's diet. The rats were monitored bi-weekly for live phase, cage side, physical and neurological parameters. The food intake and body weight was recorded twice in a week. The hematology, clinical chemistry profile, gross necropsy and histopathological profile of all organs were evaluated on day 2 (30<sup>th</sup> day) and 15<sup>th</sup> day (45<sup>th</sup> day) of post exposure to test substance. The post exposure investigations are carried out to assess reversal of unintended effects that if observed during exposure phase of test substance.

## RESULTS

### *Acute toxicity*

Mortality to the extent of 5% and 15% was recorded in mice which received 10X -DDI and 20X -DDI of test substance respectively. The autopsy report confirms that mortality was not due to test substance exposure, as no mortality was recorded in rats which received 10X -DDI and 20X -DDI concentrations of test substance.

### *Sub-chronic toxicity test*

- No mortality was recorded in any of the group.
- No significant abnormalities were recorded among five groups with reference to routine physical, physiological and neurological activities till the end of the experiment.
- Reduced food intake was observed during the 15-30<sup>th</sup> day in rats received test substance as compared to animals which received NIN standard and Vehicle control diet.
- The Gain in body weight was significantly lower in 1X DDI and 2.5 X DDI as compared to *tur dhal* (VC fed group) between 15<sup>th</sup> - 30<sup>th</sup> day of feeding. In protein restricted group (1X-DDI-PD) a significant reduction in body weight was recorded from 8<sup>th</sup>-30<sup>th</sup> day. In the post exposure phase there was no significant change in body weight gain in any group of animals.
- Blood chemistry profile including hematological parameters of groups that received test substance were significantly different from the NIN standard diet fed group.
- Histopathological study revealed various changes across all groups including Normal and Vehicle control which are common in colony bred animals.

## CONCLUSIONS

In acute toxicity tests, there was no mortality during acute exposure of test substance to the level of 20 X DDI.

In sub-chronic toxicity tests, there were no pre-terminal deaths in rats received 1X, 2.5X DDI daily for 28 days and during post exposure period of 15 days. The food intake and body weight were significantly different between groups during the end of exposure. The live phase, behavioral, clinical, neurological activity was normal throughout the study period. Though significant changes were seen in clinical chemistry and hematology profiles, the values were in normal range and cannot be attributed to the exposure to test substance. Histopathological analysis did not show any changes attributable to test substance exposed at various levels.

The oral administration of fortified red gram (*tur dhal*) daily for 28 days in maximum of 2.5 times of intended human DDI had not produced any adverse effects in rats under experimental conditions.

5

## Acute toxicity in mice and rats, sub chronic toxicity in rats and *in-vitro* allergenicity test of leaf/fruit of Bt brinjal containing Cry1Fa1 gene

Bejo Sheetal Seeds Pvt. Ltd. has licensed Bt brinjal (event 142) from Indian Agricultural Institute, New Delhi and developed Bt *brinjal* containing Cry1Fa1 gene (event 142) for insect resistant trait so as to increase the production. Since it will be recommended for human and live stock consumption and as per the bio-safety norms of DBT, acute (14 days), Sub-chronic toxicity test by feeding Bt and Non-Bt brinjal fruit daily for 90 days to rats has been carried out.

The present report contains the results of Sub-chronic investigation which has been conducted in *Sprague dawley* rats after the safety with acute exposure to pure/equivalent protein Cry1Fa1 orally in mice and rats is established.

### METHODOLOGY

**Acute toxicity test:** The safety of pure/equivalent protein Cry1Fa1 in mice & rats supplied by Bejo Sheetal Seeds Pvt. Ltd has been established at our centre with a single exposure of 2gm/kg b.wt

**Sub chronic toxicity test:** This investigation was conducted in sixty (30M+30F), *Sprague dawley* rats, aged 4- 6 weeks weighing 150 - 180g, received from the National Centre for Laboratory Animal Sciences (NCLAS) after taking approval of IAEC (Annexure- III). The animals were divided randomly into three groups viz., i) Control, ii) Non Transgenic [NT(MDI)] and iii) Transgenic [T(MDI)] and acclimatized. This was followed by training for 10 days to consume the lyophilized *brinjal* fruit powder containing transgenic and non-transgenic material in diet equivalent to MDI in addition to regular NIN pellet diet. The control group has received regular diet whereas lyophilized non-transgenic (Isogenic non-Bt hybrid) and transgenic (T) Brinjal fruit was received daily for 90 days by [NT(MDI)] and [T(MDI)] groups respectively. This was followed by monitoring the animals bi-weekly for live phase, cage side, physical and neurological parameters. The haematology, clinical chemistry profile, gross necropsy and histopathological observations of all organs and serum IgM, IgA and IgE levels were estimated on 95<sup>th</sup> day and 109<sup>th</sup> day after last exposure to test material.

## RESULTS

- No Pre-terminal deaths were recorded in animals of C, NT (MDI) and T (MDI) groups.
- No significant effect on food intake, body weight gain between NT(MDI) and T (MDI) groups
- No abnormal clinical signs, behavioral activity etc were observed in animals which received both NT (MDI) and T (MDI) groups.
- No significant changes in clinical chemistry parameters were seen.
- There were no abnormal changes in hematological parameters.
- The serum total IgE levels of NT (MDI) and T (MDI) groups were comparable to control group.
- There were no significant gross necropsy observations. The various histopathological changes seen which were common to conventional colony bred animals and also seen mostly across all groups and hence not considered significant.

## CONCLUSIONS

No pre-terminal deaths were observed in any group of animals in sub-chronic toxicity test which received test material. There were no abnormal findings with reference to gain in body weight, food intake, cage side activity and clinical observations. No abnormal clinical chemistry, hematology profile was recorded. No histopathological changes attributed to test compound were recorded. The total serum IgE was within normal range.

The feeding of Bt brinjal as a lypholised powder daily for 90 days to rats at an intake equivalent to maximum dietary intake for human, did not result in any significant changes between the non-Bt and Bt varieties and were comparable to controls fed on standard rat diet (powder form).

# LIBRARY & DOCUMENTATION SERVICES

Library continued to cater to the documentation and information needs of the Institute and other Research Organizations, Home Science and Medical Colleges. The library has played a key role in reference activities by offering information dissemination services like MEDLINE searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL<[http://groups.yahoo.com/group/ICMR Librarians](http://groups.yahoo.com/group/ICMR_Librarians)>.

The Library has continued to provide an excellent photostat support to the scientists, technical as well as to the administrative staff. Resource sharing and User Education Programmes etc., are continuously being undertaken by the library. Institute's Scientific papers are going in for publication in Scientific Journals etc., are being routed through the library and a data-base of the published papers is also made accessible through on-line services using NIN Website ([www.ninindia.org](http://www.ninindia.org)).

## Modernisation of Library and Information Network

The following work was taken up and the equipments were procured for strengthening the services of dissemination of information to the scientists.

- a) ICMR has renewed the subscription to Proquest Medical Library Full Text Database of the journals. During the period a total of 2690 Proquest ML Full Text Database Searches were made.
- b) Subscription of JCCC@ICMR and J-Gate has been renewed by Indian Council of Medical Research through M/s. Informatics India Pvt. Ltd., Bangalore, JCCC@ICMR covers more than 1679 journals received collectively at 29 Institutions/Centres Consortia of ICMR Libraries. And J-Gate is an electronic gateway to global e-journals literature. It presently has massive database of journal literature indexed from more than 27,672 e-journals with links to full text at publisher sites and provides free access to full-text of 2633 journals with e-author e-mail address and also one can find the availability of the journal in a local library.
- c) NIN Library is also a member of NML - ERMED Consortia for accessing 1812 Journals
- d) Online subscription of 5 Core journals such as BMJ, LANCET, NATURE, NEJM and SCIENCE was renewed by ICMR is also accessible.
- e) The following equipments were procured for the library.
  - i) HP PC - 5
  - ii) UPS - 5
  - iii) HP Laserjet Printer - 1
  - iv) Barcode Scanners - 4

### NEW JOURNALS ADDED

#### Indian Journals

Nanotech Insights Newsletter

The following library services were expanded as detailed below:

#### 1. New additions

Books	....	182
Reports	....	245

# library & documentation services

Journals (New Subs.)	....	1
Thesis / Dissertations	....	9
CDROMS	....	16
PC Quest CD's ....		12
General CD's ....		4
<b>2. Other activities</b>		
Journals Bound	....	1,888
Visitors using the Library	....	2, 239
Circulation of Books/Journals etc	....	1, 279
No. of E-mails sent outside	....	1, 800
No. of E-mails received	....	15, 615
Photocopying ( No. of pages )	.....	3,05,846
Number of Annual Reports mailed	....	520
No. of INTERNET Searches provided	....	200
No. of Reprints sent	....	50
Proquest Full Text Database searches provided	....	2,690
<b>3. Total library collections</b>		
Books	....	17,806
Journals (Bound Volumes)	....	35,623
Journals subscribed for 2010	....	357
Journals received (Gratis/Exchange)	....	320
Microforms (Microfiche)	....	1,080
Slides	....	280
Reports	....	13,161
Theses & Dissertations	....	387
MEDLINE CDROMS Discs	....	383
Current Contents on Diskettes with abstracts	....	664
Proquest (Full Text E-Journals) on CD ROMS	....	495
General CD's	....	210

## Ph.D PROGRAMMES

### Ph.D Awardees

S. No	Research Scholar	Title of the thesis	Award Year	University
1	Y Srinivasa Reddy	Effect of environmental lead exposure on infection and immunity in under nutrition	2011	Osmania
2	Prashant A	Impact of various doses of vitamin A on obesity and lipid metabolism in N/Ob rats	2011	Osmania
3	Md. Naseeruddin	Understanding the role of micronutrients in pathogenesis of <i>Mycobacterium tuberculosis</i>	2011	Osmania
4	Shashikiran G	In vitro regeneration of the insulin secreting cells from the adult pancreatic ductal epithelial cells (progenitors/stem cells) - the role of specific nutrients	2011	Osmania
5	Rajukumar D	Characterization and differentiation of pancreatic progenitor/ stem cells (Nestin positive cells) to insulin secreting cells - the role of specific nutrients	2011	Osmania
6.	Sylvia Fernandez Rao	Effect of maternal self - esteem and locus of control on the receptivity to intervention on responsive feeding and their impact on growth and development of rural infants	2011	Osmania

### Research scholars registered for Ph.D

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
1.	Manisha Ganeshan (2005)	Foetal origins of adiposity and insulin resistance : Role of peri/postnatal manganese status	Dr. Raghunath M	Osmania <i>Submitted thesis 2011</i>
2.	Vara Prasad SSS (2005)	Exploration of basal glucocorticoid and levels and their possible role in obesity and insulin resistance using WNIN/Ob and WNIN/GR-Ob rat models	Dr. Vajreswari A	Osmania <i>Submitted thesis 2011</i>
3.	Sainath PB (2005)	Insulin, insulin receptor and its signaling mechanism(s) in the brain and insulin sensitive target organs in the WNIN-Ob and WNINGR- Ob rats	Dr. Raghunath M	Osmania <i>Submitted thesis 2011</i>

# ph.d programmes

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
4	Anand Kumar K (2006)	Maternal vitamin B <sub>12</sub> restriction induced changes in body adiposity, hyperglycemia and insulin resistance in WNIN rat offspring : Molecular basis of the changes	Dr. Raghunath M	Osmania <i>Submitted thesis 2011</i>
5.	Pratibha B (2005)	Immune status of WNIN mutant rats with reference to leptin and obesity	Dr. Giridharan NV	Osmania
6	Sreevani M (2005)	Understanding and dissecting the role of resistin in the etiology of insulin resistance using obese rat model	Dr. Nasreen Z Ehtesham	Osmania
7	Priyanka Shankar (2006)	Studies on high fluoride and low calcium on bone metabolism in rats: Biochemical mechanisms	Dr. AL Khandare	Osmania
8	Little Flower Augustine (2007)	Stress allostatic load and micronutrient status among higher secondary students: Impact of dietary advice	Dr. Madhavan Nair K	Osmania
9	P Muthenna (2007)	Characterization of active principles of aldose reductase and antiglycating agents in functional foods	Dr. Bhanuprakash Reddy G	Osmania
10	A. Satyanarayana (2007)	Biochemical, molecular and nutritional aspects of diabetic retinopathy	Dr. Bhanuprakash Reddy G	Osmania
11	Agatha Betsy (2008)	Assessment of dietary exposure to select contaminants and dietary intake of select nutrients among the various socioeconomic sections of Hyderabad	Dr. Kalpagam Polasa	Osmania
12	Prathipati Vijayakumar (2008)	Role of polyunsaturated fatty acids (PUFAs) on regulation of obesity using an obese mutant rat model (WNIN/Ob)	Dr. Vajreswari A	Osmania
13	Soundarya (2008)	Establishment of preproliferative cell lines from adult adipose tissue of WNIN mutant rats (WNIN GR-Ob and WNIN Ob/Ob)	Dr. Vijayalakshmi V	Osmania
14	Swarnim Gupta (2008)	Dietary diversification of Indian vegetarian diet to improve the iron bioavailability: Studies using CaCo-2 cell model	Dr. Madhavan Nair K	Osmania

# ph.d programmes

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
15	Deethu Sarah Varghese (2008)	Assessment of body composition in Indian females using different techniques	Dr.Venkatramana.Y	Osmania
16	B ShankarAnand (2009)	Role of T cells and secreted cytokines in insulin resistance and obesity	Dr. Sudeep Ghosh	Osmania
17	Ramesh Athe (2009)	Meta analysis approaches on "Micronutrient food fortification and its effect on health, social and economic factors" - A statistical model building	Dr. Vishnuvardhana Rao M	Osmania
18	Chetan C. Nimgulkar (2009)	Evaluation of herbs/nutraceutical products for anti atherosclerotic agent	D Dinesh Kumar B	Osmania
19	Mehraj-Ud-Din Bhat (2009)	Role of UPP in vitamin D deficiency induced muscle atrophy and hypoinsulinemia	Dr. Ayesha Ismail	Osmania
20	A. Gandhi Nayak (2009)	Vitamin A metabolism in obesity	Dr. Vajreswari A	Osmania
21	Anupama Tyagi (2009)	Anti-inflammatory potential of n3 PUFA in experimental ulcerative colitis: Biochemical and molecular study	Dr. S. Ahmed Ibrahim	Osmania
22	V. Sudhakar Reddy (2009)	Role of small heat shock proteins in diabetic complications	Dr. Bhanuprakash Reddy G	Osmania
23	Pallavi Namburi (2010)	Regulatory role of zinc in Hepcidin mediated iron metabolism	Dr. Madhavan Nair K	Osmania
24	N Bindu (2010)	Effect of polyphenol action on cancer cell	Dr. Ayesha Ismail	Osmania
25	Jitendra Kumar (2010)	IGF-1 and BDNF signaling in the brain of WNIN Obese mutant rats during ageing: Effect of calorie and micronutrient restrictions	Dr. Raghunath M	Osmania
26	Anju Elizabeth Thomas (2010)	Fetal programming for neuro - muscular skeletal development in the rat offspring - Role of antenatal and perinatal Mg deficiency.	Dr. Raghunath M	Osmania
27	Himadri Singh (2010)	Establishment of propagable cell lines from pancreas (Ductal Epithelial Cells) from WNINGR - Ob rats	Dr. Vijayalakshmi V	Osmania
28	Sarin Sara Jose (2010)	Diabetic complications: Dietary intake and blood levels of nutrients	Dr. Bhanuprakash Reddy G	<i>To be registered</i>

# ph.d programmes

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
29	Anil S (2010)	Modulation of adipose tissue inflammation by dietary n-3 PUFA	Dr. S. Ahmed Ibrahim	To be registered
30.	Y. Sravanthy (2010)	Effect of prenatal iron supplementation on iron-zinc homeostasis and placental zinc transporters. Studies in pregnant women and in iron/zinc depleted Be Wo cell line.	Dr. Madhavan Nair K	To be registered
31	Mr. Chalamaiah (2010)	Fish Egg Protein Hydrolysis as Nutraceuticals /Health food in promotion of immuno-modulatory activities	Dr. B. Dinesh Kumar	To be registered
32	Mrs.S.Madhuri (2010)	-	Dr.Nasreen Z.E.	To be registered
33	Mr.K.Ashok Reddy (2010)	-	Dr.P.Uday Kumar	To be registered
34.	Mr.Madhoosudan Ananth Patil (2010)	Studies on diabetic complications: Evaluation of suitable animal models and role of dietary agents	Dr. P.Suryanarayana	Osmainia
35.	Mr.G.Venkateswarlu (2010)	-	Dr.Sudip Ghosh	To be registered
36	Mr.Sandeep Kumar (2011)	-	Dr.Sudip Ghosh	To be registered
37	P Ravindranath (2011)	Purification characterization and primary elucidation of human milk factor that enhances iron absorption	Dr. P. Raghu	To be registered
38	A.Vijayendra Chary (2011)	Molecular link between Cd23 and Cd21 expression and role of infant nutrition in young children with allergic asthma	Dr.B.Dinesh Kumar	To be registered
39	Ms.V.Varsha (2011)	-	Dr.B.Dinesh Kumar	To be registered
40	Mr. Nagabhushan Reddy (2011)	Anti diabetic effects of some simple amino acid-chromium complexes and their probable mechanism of action	Dr. M. Raghunath	To be registered
41	Ms.Shampa Ghosh (2011)	-	Dr.M.Raghunath	To be registered
42	Mr.V.Anantha Krishna (2011)	-	Dr. A. Vajreswari	To be registered
43	Ms.Ch.Anuradha (2011)	-	Dr. A. Vajreswari	To be registered
44	Ms.K.Sowmya (2011)	-	Dr.Vijayalakshmi	To be registered
45	Ms.J.Vahini (2011)	-	Dr.K.Bhaskara Chary	To be registered
46	Ms.Naveena (2011)	-	Dr.K.Bhaskara Chary	To be registered

# ph.d programmes

S. No	Research Scholar (Year of joining)	Title of the thesis	Supervisor	University
47	Mr.Nagamuralidhar (2011)	-	Dr.Rajender Rao	To be registered
48	Ms.ChViday (2011)	-	Dr.Rajender Rao	To be registered
49	Mr.M.Ankulu (2011)	-	Dr.A.L.Khandare	To be registered
50	Ms. N. Aparna (2011)	-	Dr.A.L.Khandare	To be registered
51	Mr.A.Kiran Kumar (2012)	-	Dr.K.Madhavan Nair	To be registered
52	Mr.M.Purna chandra (2012)	-	Dr.P.Raghu	To be registered
53	Mr.S.Vishwaraj (2012)	-	Dr.Bhanu Prakash Reddy	To be registered
54	Ms.Daniella Anne L.Chyne (2012)	-	Dr.R.Ananthan	To be registered
55	Ms.M.Srujana (2012)	-	Dr.Padmaja	To be registered
56	Ms.Alekhya (2012)	-	Dr.V.Sudershan Rao	To be registered

## Institutional staff registered for PhD

S. No	Name of the Staff	Title of the thesis	Supervisor	University
1.	Dr. A Laxmaiah (2008)	Assessment of prevalence of overweight/obesity, hypertension and type II diabetes among 20 - 60 year urban population in Hyderabad	Dr. B Sesikeran	Dr. NTR University of Health Sciences
2.	P.Yadagiri Reddy (2007)	Molecular studies on obesity - Induced cataractogenesis	Dr. G. Bhanu prakash Reddy	Osmania Submitted thesis 2011

## AWARDS/ HONOURS CONFERRED ON SCIENTISTS

Name of the scientist	Award/ Honour received
Dr.GM.Subba Rao	Selected to the Board of Editors of "The Journal of Nutrition Education and Behaviour - An Elsevier Journal".
Dr.K.Bhaskarachary	"2011 SAB Award of Excellence in Food Safety and Food Security" and Fellow Award 2011 by the Society for Applied Biotechnology, Tamilnadu, India.
Dr.K.Madhavan Nair	Fellow of Academy, National Academy of Sciences (India).
Dr.B.Sesikeran	23 <sup>rd</sup> Srikantia Memorial Oration Award during the 43 <sup>rd</sup> National Conference of the Nutrition Society of India

### Awards/ Honours conferred on Research Fellows/ Students

Name of the student	Award/ Honour received
Dr. I.J.N. Padmavathi (Research Fellow)	Young Scientist Award by A.P. Akademi of Sciences.
Mr. Chetan Nimgulkar (Research Fellow)	Govind Achari Prize for the paper entitled "In silico & In vivo validation of Herbal and Nutraceutical combination as Anti - inflammatory agent", in 44 <sup>th</sup> Annual Conference of IPS held at Manipal (Dec.19-21, 2011).
Mr. Venugopal Racha (Research Fellow)	Best poster presentation for "Varies in Carrageenan inflammatory profile of different species", in 44 <sup>th</sup> Annual Conference of IPS held at Manipal (Dec.19-21, 2011).
<i>The following Research Fellows and students (MSc) received awards for posters presented at the 43<sup>rd</sup> National Conference of the Nutrition Society of India, held at NIN</i>	
Ms.Roy Choudhury Dripta (Research Fellow)	Best poster award for Dietary iron density and prevalence of anaemia among adolescents of government residential schools in Hyderabad ( <i>Community Nutrition</i> )
Ms.S.Alekhya (MSc [AN] student)	Microbiological quality of salads served along with street foods in Hyderabad ( <i>Community Nutrition</i> )
Ms.B.Swetha (MSc [AN] student)	Risk perceptions on food safety issues among women - A study ( <i>Community Nutrition</i> )
Ms. Swarnim Gupta (Research Fellow)	Dr.K.Seetharam Bhat Memorial prize for paper entitled Development of rapid and sensitive method for screening iron bioavailability in food samples ( <i>Experimental Nutrition</i> )
Ms.N.Bindu (Research Fellow)	Murraya Koenigil leaf extract inhibits proteasomal activity and retards cancer cell growth ( <i>Experiemntal Nutrition</i> )

## PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS/ CONFERENCES/ WORKSHOPS

Date	Name of the Scientist	Conference/ Meeting/ Workshop/ Seminar/ Training
2011		
May 22- June 4	Dr. A.Laxmaiah	Training Programme on Basic genetics and genomics and Genetic association studies and participated in the interactive sessions with the scientists of Bristol University and London School of Hygiene and Tropical Medicine, UK
April 25-May 6	Dr.G.Bhanu Prakash Reddy	Visited University of Michigan for the training on "Small Animal Electroretinography (ERG)" Annual Meeting of Association for Research in Vision and Ophthalmology (ARVO) held at Fort Lauderdale, Florida (USA). Presented papers on "Characterization of retinal degeneration in a novel obese rat model" and "Diabetic Retinopathy: Association with Hyperhomocysteinaemia and Vitamin -B <sub>12</sub> Deficiency" at the Indo-US workshop on Vision Research
June 5-8	Dr.I.I.Meshram	7 <sup>th</sup> Asia Pacific Conference on Clinical Nutrition, at Bangkok, Thailand. presented a paper on "Prevalence of overweight/obesity, hypertension and its correlates among adult tribal population in select States of India"
June 5-8	Dr.M.S.Radhika	7 <sup>th</sup> Asia Pacific Conference on Clinical Nutrition, at Bangkok, Thailand. presented a paper on "Bio-effect of iron fortified rice on body iron stores: A double blind randomized, placebo -controlled mid-day meal feeding trial in Indian school children"
July 13-17	Dr.GM.Subba Rao	International Association for Media and Communication Research (IAMCR 2010) at KhadirHas University, Istanbul, Turkey. Presented a poster titled "Communicating nutrition information in community settings- a critical examination of some institutional approaches in India" and co-chaired at technical session in the Health Communication Working Group of the conference.
Nov. 16-18	Dr.K.V.Radhakrishna	33 <sup>rd</sup> Session of Codex Committee on "Nutrition and Foods for Special Dietary Uses", held at Bad Soden am Taunus, Germany
December 1-18	Dr.V.Sudershan Rao	Served as International Consultant (Food Safety) for FAO Bangladesh Food Safety Project

# participation of scientists in intl. meetings/conferences/workshops

Date	Name of the Scientist	Conference/ Meeting/ Workshop/ Seminar/ Training
<b>2012</b>		
Feb. 7	Dr.B.Sesikeran	One day seminar on "Food Safety Assessment for Members of the Technical Committee" organized by South Asia Biosafety Programme, at Bangladesh. Delivered a talk on "Biosafety issues and guidelines and principles".
March 1-3	Dr.Bharathi Kulkarni	International Conference on "Nutrition and Growth", held at Paris, France. Delivered a talk on "Early nutrition intervention and lean body mass development in young Indian adults".

## WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN

1. National Workshop on “Next Generation Sequencing”, organized by Bio-Medical Informatics Centre, ICMR, New Delhi (June 13-17).
2. Training Workshop on “Role of Risk Analysis in the Development and Implementation of Food Safety Programmes and Standards”, organized by Biotech Consortium Limited, University of Nebraska-Lincoln and University of Maryland, USA (June 20-22).
3. One day Seminar on “National Priorities in Nutrition Research” and Release of Revised Dietary Guidelines for Indians - A Manual, organized by National Institute of Nutrition and Federation of All India ICMR Employees (July 4).
4. Training of Trainers for “National Programme for Prevention and Control of Fluorosis” organized by National Institute of Nutrition in association with Directorate General of Health Services, Ministry of Health, Government of India, New Delhi (July 28-29).
5. Meeting of the Scientific Advisory Committee of NIN/ FDTRC/ NCLAS (Aug. 17-19).
6. NAMS Golden Jubilee Regional Symposium - 2011 on “Recent Advances in the Prevention and Management of Non-Communicable Diseases” (Aug. 26).
7. State level Workshop for DEOs and MEOs of Andhra Pradesh on “Healthy Diets and Lifestyles” organized in association with World Health Organisation, India (Aug. 29-30).
8. An Indo-UK Workshop on “Infant and Child Growth Trajectories and the Developmental Origins of Adult Chronic Diseases in India” was organized in association with Department of Science and Technology, Government of India and The Royal Society, London (Sept. 8-10).
9. XXXIX Annual Training Course on “Endocrinological Techniques and their Application”. About ten candidates from different States of the country participated in the course (Sept. 19 – Oct. 28, 2011).
10. In connection with the World Food Day celebrations, a one day symposium was organized on “Food prices – From crisis to stability”, by Association of Food Scientists and Technologists, Hyderabad Chapter and Oil Technologists Association of India (Oct. 16).
11. Brainstorming Meeting on “Status of Food Allergy in India” (Oct. 31).
12. Nutrition Society of India Pre-Conference Workshop on “Methodology of Nutrition Assessment and Body Composition” and “Concepts, Principles and Applications of Nutrigenomics” (Nov. 10).
13. 43rd National Conference of Nutrition Society of India on the theme “Economic Transition in Nutrition – Lifestyle Diseases and Health and Nutrition Wellness” (Nov. 11-12).
14. Brainstorming Session on “Fighting Child Malnutrition”, organized jointly by National Academy of Agricultural Sciences, New Delhi and Nutrition Society of India (Nov. 13).
15. Workshop and Training Program on “Sampling and Detection Methods Applied to Transgenic Crops”, organized in association with ILSI-India and ILSI International Food Biotechnology Committee, Washington DC, co-sponsored by Department of Biotechnology, Ministry of Science and Technology, GoI and Food Safety and Standards Authority of India (Nov. 17-19).<sup>1</sup>
16. Seed Division<sup>1</sup> Group Monitoring Workshop organized in association with Science and Technology Component for Women, Department of Science and Technology (Nov. 23-24).

## workshops/conferences/seminars/ training programmes

17. Joint Workshop on “Improving Nutritional Health of Under 5yr Children of Mobile and Migrant Communities” organized in association with Dr.Reddy's Foundation, Hyderabad (Nov. 24).
18. Forty ninth Post-Graduate Certificate Course in Nutrition (Jan. 4 - March 16, 2012). About thirteen students from different states of the country attended the course.
19. A Training programme was organized on “Assessment of Health and Nutrition” for the Field staff of CBM-Canada Non-governmental organization, by the Division of Community Studies (Jan. 30 - Feb. 1).
20. Workshop on “Qualitative Health Research”, National Task Force Study on Migrants Health Care, organized in association with Health Systems Research Division, ICMR, New Delhi (Feb. 15-17).
21. Meeting of the ICMR Tribal Health Forum. The meeting was chaired by Dr.V.M. Katoch, DG, ICMR and Directors and senior scientists from various ICMR institutes participated and appraised of the progress of research activities related to health of tribal populations (March 4).

# SERVICES RENDERED TOWARDS INCOME GENERATION

## 1 PATHOLOGY SERVICES

During the year, a total income of ₹ 2,46,540/- was generated from various projects of Institute's pre-clinical toxicology and surgical pathology and cytology samples analyzation.

## 2 TRAINING PROGRAMMES

- I. An amount of ₹ 6,20,000/- was generated from the tuition fee collected from the first and second year participants of 2 year MSc (Applied Nutrition) course (1<sup>st</sup> year – 16 and 2<sup>nd</sup> year – 15 candidates).
- ii. An amount of ₹ 1,42,000/- was generated from fifteen private candidates admitted to the regular training programme viz., Post Graduate Certificate Course in Nutrition (11) and Annual Certificate Course on Endocrinological Techniques and their Applications (4).

## INSTRUMENTATION SERVICES

### List of equipments procured & installed during the financial year 2011-12

Sl. No.	Instrument	Make
1	Quat. Grad. HPLC with PDA & FLS Detector	Dionex
2	Mini Electrophoresis and Blotting Unit	Genetix Biotech
3	3 KVA UPS	APC
4	3 KVA UPS	APC
5	Orbital Shaker	IKA
6	Computerized Gel Documentation and Analysis System	Expert Vision
7	Vacuum Pump	Promivac Engineers
8	Vortex Mixer	Fisher Scientific
9	Orion pH Meter	Thermo Scientific
10	Electro Blot Transfer Assembly & Power Supply	Merck - Ge Nei
11	Atomic Absorption Spectrophotometer with Graphite Furnace	Shimadzu
12	Gas Chromatograph with Head Space Auto Sampler	Perkin Elmer
13	Binary gradient HPLC with PDA and FLD.	Dionex
14	Binary gradient HPLC with PDA and ELSD.	Dionex
15	Hot plate with magnetic stirrer	IKA
16	Centrifuge	Sigma
17	Binocular stereo Microscope	Olympus
18	pH meter	Orion
19	Table top Centrifuge	Kubota
20	Vacuum Pump	Promivac
21	Rotary Flash Evaporator	Heidolph
22	Autoclave	KK Scientific
23	PH Meter	Global
24	Homogenizer	IKA
25	Dry Heating Block	WISWO
26	Electrophoresis	Biorad
27	Co2 Incubator	Thermo
28	Co2 Incubator	Thermo
29	Gel Rocker-2No	Genei
30	Table top Centrifuge	Thermo

# INSTRUMENTATION SERVICES

Sl. No.	Instrument	Make
31	Maldi ToF/Tof-MS/MS	5800 TOF/TOF
32	RT-PCR	CFX96
33	Bio-Rad Protein Purification System	Biologic Duoflow Quad Tec10 & Bio fraction Collector
34	Organic Flash Purification System	Combiflash Rf200
35	Off-Line 0.5 -2nos.	Microtek
36	2 On-Line System -1no.	Digitalo Microprocessor IGBT
37	Multispin 4 position motorless Magnetic Stirrer -1no.	4060
38	Vortex Mixers-4nos.	SA8/1
39	Top Loading Balance-1no	GE7101-1
40	Photo Copier-1no	AR-M205
41	Agilent Electrophoresis Bioanalyzer	2100G2939A
42	Agilent LCMS Single Quad+ QPCR+ Agilent 1200 LC	G4277A 401513
43	Heating Block with Stirring Option	Reacti Therm III
44	Indirect Ophthalmoscope	Vantage Plus
45	Electroretinogram	UTAS
46	Gas Chromatograph	Calrus 680

## NIN SCIENTIFIC PUBLICATIONS – 2011

### A Papers published in scientific journals

1. Abhaykumar, Nirmala K, Prasad MPR, Panpatil VV, Prasanna Krishna T, Sesikeran B, Kalpagam Polasa : Reduction in platelet aggregations (in vitro) by diallyl sulphide in female participants with type 2 diabetes mellitus. *J Pharmacol Toxicol.* 6 : 381-390, 2011.
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5. Arlappa N, Laxmaiah A, Balakrishna N, Harikumar R, Mallikarjuna Rao K, Gal Reddy Ch, Saradkumar S, Ravindranath M, Brahmam GNV : Micronutrient deficiency disorders among the rural children of West Bengal, India. *Ann Hum Biol.* 38 :281-289, 2011.
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16. Devindra S, Longvah T : A new approach for the measurement of digestible carbohydrates in different food samples with HPLC-RI. *J Agric Sci Tech.* B 1:1216-1223,2011.
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## B Papers published in proceedings/books

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2. Arlappa N, Gangadhar, T, Laxmaiah A, Brahmam GNV. Endemic Kidney Disease among Population residing in Mica belt of Andhra Pradesh, India. 16th World Congress on Clinical Nutrition. Sept 11-14, 2011. New Delhi.
3. Bhanuprakash Reddy G: Concepts, principles and applications of nutrigenomics. In "Pre-conference workshop 43rd National Conference of Nutrition Society of India, Hyderabad", November 10, 2011, pp.4-6. (Abstract)
4. Bhanuprakash Reddy G: Micronutrient status in diabetic retinopathy. In "9th Annual meeting of Indian Eye Research Group", 30th-31st, July 2011 (Abstract)
5. Bhanuprakash Reddy G: Molecular-target based phytonutrients for diabetic complications, Symposium (Molecular Nutrition) In "80th Annual Meeting of Society of Biological Chemists (India)", Lucknow, November 12-15, 2011, p.216, (Abstract)
6. Bhanuprakash Reddy G: Mutations, retinal dystrophies and phytonutrients, symposium (Chemical Basis of Nutrient Function). In "43rd, National Conference of Nutrition Society of India, Hyderabad", November 11-12, 2011, p.44 (Abstract).
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8. Bhanuprakash Reddy G: Structural and functional impact of a novel mutation (F71L) in alpha A-crystallin associated with age-related cataract. In "The Indo-US Bilateral symposium on Aging and Age-related Diseases, National Institute of Immunology, New Delhi, Mar 3-4, 2011 p.12 (Abstract).
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13. Hurley K, Fernandez Rao S, Vazir S. Nair KM, Balakrishna N, Sesikeran B, Harding K, Reinhart G, Black M. Designing an intervention to promote child development among

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  23. Laxmaiah A: Non-communicable diseases, special focus on childhood obesity and preventive strategies by National conference on "Nutrition in metabolic disorders" organized by Centre for Nutrition Counseling, 10th & 11th October 2011.
  24. Laxmaiah A : Prevention and control of Non-communicable diseases in school children. 9th Madras Diabetic Research Foundation (MDRF), Chennai, India and University of Alabama (UAB), USA International Seminar on Prevention & Control of Non-communicable diseases,

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