

# RESEARCH HIGHLIGHTS

## 1. COMMUNITY STUDIES

### 1.1 Developing a multi-component nutrition and health education intervention model to combat persistent problem of undernutrition in the rural areas of Allahabad, Uttar Pradesh

The prevalence of undernutrition among <5 year children was alarmingly high. Therefore, a multi-component nutrition education intervention district model to combat the persistent problem of undernutrition was developed in the district of Allahabad, Uttar Pradesh'. Phase I study (formative research: quantitative and qualitative) was completed. Based on the formative research findings, the development of sustainable nutrition intervention strategies and piloting of the strategies are in progress.

### 1.2 District level mapping of undernutrition among under 5 years children, adolescent girl, pregnant woman and lactating mothers and infant & young child feeding practices of mothers of under 3 years children in the state of Andhra Pradesh and Pudhucherry

Even though the prevalence of undernutrition among <5year children is declining throughout the country, the current prevalences of undernutrition is still at alarming levels, when compare with figures of developed countries. Therefore, district level mapping of undernutrition and its determinants was done to enable planners/implementers to develop area specific intervention strategies and programmes for prevention and control undernutrition. District level mapping of undernutrition was earlier done in the states of Madhya Pradesh, Gujarat, Meghalaya, Haryana and these states had already initiated area specific interventions. Similarly, district level mapping in the states of Andhra Pradesh and Pudhuchery was completed and results have been published as a report. Respective state governments can now initiate area specific interventions in their states to control undernutrition.

### 1.3 Assessment of magnitude, prevalence and aetiology of Chronic Kidney Disease (CKD) among the rural population residing in Mica belt of Nellore District, Andhra Pradesh.

Several news reports and hospital records revealed that reported cases of CKD patents were high in the district of Nellore, where Mica belts exist. A study was carried out to assess magnitude, prevalence and etiology of CKD among the rural population in the Mica belt areas of Nellore district Andhra Pradesh at different time points. As per the recommendations of NIN, the ground water (mica/silica) was replaced with surface water (Khandaleru Reservoir), subsequently serum creatinine levels significantly declined and the prevalence of CKD also significantly declined over a period of time.

## 2. MICROBIOLOGY AND IMMUNOLOGY

### 2.1 A prospective cohort study to understand periconception and prenatal factors that influence fetal & postnatal growth and development

Nine hundred and twenty eight (928) pregnant women were recruited at 20-24 weeks gestation and were followed through during pregnancy uptill birth. Low birth weight (LBW)and Preterm births (PTB) were prevalent in 22.1% and 7.1% respectively. The mean ( $\pm$ SD) birth-weight was 2,610 $\pm$ 46 g. When less than 10% birth-weight for gestational age of a reference population was used as a proxy for fetal growth restriction (FGR), 33% neonates had FGR. Maternal weight (Mwt),

Mid Upper Arm Circumference ( MUAC) and skin fold at four sites were collected during 22-24 weeks of gestation, 30-34 weeks of gestation and more than 36 weeks of gestation. In a linear regression model, mid upper arm circumference (MUAC), both early and late in pregnancy, was significantly associated with birth-weight, maternal age, maternal weight and maternal skin fold thicknesses at all four sites – Biceps, triceps, subscapular, suprailiac. Low maternal MUAC (<24.0 cm) in either early or late pregnancy was associated with an increased risk of low birth weight (LBW) and fetal growth restriction (FGR). MUAC <24.0 cm had 2 fold increased risk of LBW (odds ratio [OR]=2.985; p= 0.035) or FGR (odds ratio [OR]=2.984; p= 0.019). Maternal wasting, reflected by a single low MUAC (<24.0 cm) during pregnancy, is associated with a 2-fold higher risk of LBW. A single MUAC provides a simple, inexpensive and reliable approach to identify mothers at high risk of bearing an LBW infant and even greater risk of having a growth-restricted infant.

### 3. BASIC STUDIES

#### 3.1 Isolation and characterization of food derived iron binding peptides and their effect on iron bioavailability: A proteomic approach.

Binding and solubilization of ferric iron by food peptides, released during digestion, facilitate intestinal iron absorption. In the present study, release of iron-binding peptides during *in vitro* gastrointestinal digestion of chicken egg white and buffalo milk was investigated. The iron-binding activity of the egg white protein and buffalo milk increased upon gastrointestinal digestion. The iron-binding activity from the digests was purified by gel filtration chromatography followed by reverse phase HPLC. Subsequently, the peptide sequences were characterized by MALDI-MS based methods. The egg white peptide (DKLPGFGDS(PO<sub>4</sub>)IEAQ, 1456.7 amu) was identified as an internal fragment of ovalbumin while the milk peptide (MHQPPPQLPPT, 1242.56 amu) was identified as an internal fragment of  $\beta$ -casein. The synthetic peptide corresponding to the identified egg white iron-binding peptide bound and increased <sup>59</sup>Fe-iron uptake. However, the milk peptide, although bound iron, inhibited the absorption of iron in intestinal Caco-2 cells. Further, dephosphorylation of egg white synthetic peptide completely inhibited the iron-binding activity, while methyl-esterification of its carboxyl groups partially inhibited the activity. These results suggest that food derived peptides modulate intestinal iron absorption and that the isolated iron-binding egg peptide could be explored further as a potential nutraceutical for improving iron absorption.

#### 3.2 Role of Vitamin D in adiposity

The aim of the present study was to examine the role of vitamin D and calcium on body adiposity in a diet-induced vitamin D deficient rat model. Vitamin D-deficient rats gained less weight and had lower amounts of visceral fat. Consistent with reduced adipose tissue mass, the vitamin D-deficient rats had low circulating levels of leptin, which reflects body fat stores. Expression of vitamin D and calcium sensing receptors, and that of genes involved in adipogenesis such as peroxisome proliferator-activated receptor, fatty acid synthase and leptin were significantly reduced in white adipose tissue of deficient rats compared to vitamin D-sufficient rats. Furthermore, the expression of uncoupling proteins (Ucp1 and Ucp2) was elevated in the white adipose tissue of the deficient rat indicative of higher energy expenditure thereby, leading to a lean phenotype. Expression of the p160 steroid receptor coactivator 3 (SRC3), a key regulator of adipogenesis in white adipose tissue was decreased in vitamin D-deficient state. Interestingly, most of the changes observed in vitamin D deficient rats were corrected by calcium supplementation alone. Our data demonstrates that dietary vitamin D and calcium regulate adipose tissue function and metabolism.

### **3.3 Amino acid – metal complexes as model for the glucose tolerance factor of yeast: hypoglycaemic activity and therapeutic potential in diabetes; synthesis, structure and mechanism of action in yeast and animals**

Our attempt to assess the utility of oral administration of simple, binary complexes of Cr(III) with amino acids in the treatment of Diabetes, indicated that like Cr-D-Phe<sub>3</sub> complex, complex of Cr(III) with L-Phe but not Gly or L-Cys, was effective in alleviating all the ill effects of High Sucrose feeding on glucose tolerance, intracellular metabolism and plasma lipid profile and mitigation of the associated changes at various levels of intracellular insulin signalling pathway, expression of key regulatory enzymes of metabolic pathways and changes in oxidative stress/anti oxidant status. The data suggests that binary complexes of Cr with only a few (but not all) amino acids may be effective in alleviating high sucrose diet induced insulin resistance/T2DM in male Sprague Dawley rats.

### **3.4 Molecular basis of maternal vitamin B12 restriction induced changes in the C57BL/6 mouse offspring: Role of epigenetics**

Severe vitamin B12 deficiency in C57BL/6 female in addition to altering the body composition, lipid profile and reproductive performance also altered the behaviour of the F0 mice which appeared to be anxious and depressed and altered epigenetics appear to underlie their etiopathology. Transgenerational vitamin B12 deficiency of both severe and moderate intensity also altered body composition, induced dyslipidemia, fasting hyperglycemia, insulin resistance in addition to inducing anxiety and depressive behaviour in F1 offspring. In offspring also, increased inflammation, stress and impaired antioxidant status were associated with the deleterious effects of vitamin B12 deficiency. While rehabilitation from weaning appeared ineffective in alleviating maternal vitamin B12 deficiency induced changes in offspring, rehabilitation of B12R mothers from parturition could only delay but not prevent the onset of the deleterious changes in the offspring in general. Genome-wide gene expression studies suggest that modulation of some important signaling pathways involved in the development and function of the brain may underly the alterations observed in the offspring born to vitamin B12 restricted mice.

### **3.5 Status of vitamin B12 and folate among different adult groups**

Deficiencies of vitamin B12 (B12) and folate (FA) lead to a wide spectrum of disorders that affect all age groups. Hence, to determine the plasma levels and dietary intake of B12 and FA in the adult population, a community-based cross-sectional study was conducted. The study also looked into dietary intake of B12 and FA in an urban setup among apparently healthy adults distributed into three age groups: 21-40, 41-60 and >60 years. The overall prevalence of FA deficiency was 12%, and there was no significant difference in plasma FA concentrations among the groups. While the overall prevalence of B12 deficiency was 35%, it was significantly higher in the 21-40 (44%) and 41-60 age groups (40%) when compared with the >60 group (30%). B12 deficiency was higher in vegetarians (54%) compared to those consuming mixed diet (31%), and the reverse was the case with FA. However, the dietary intakes of FA and B12 were not significantly different among the groups.

### **3.6 Development, validity and reproducibility of a raw food based quantitative food frequency questionnaire (RFQnFFQ)**

Food frequency questionnaire (FFQ) is a commonly used tool to assess long-term habitual dietary-intake pattern related to chronic diseases. An attempt was made to develop a 141 item raw food based quantitative food frequency questionnaire (RFQnFFQ) based on commonly consumed foods

and local food habits. The pilot tested RFQnFFQ was validated against the standard 24-hour dietary recall (24hR) method. The reproducibility of RFQnFFQ was assessed at two reference periods in a year including seasonal variation. The reproducibility of the RFQnFFQs was found to be good and no significant seasonal difference in food and nutrient intake was observed by the RFQnFFQ. The agreement between the RFQnFFQ and 24hR validated the utility of RFQnFFQ. The RFQnFFQ *of one year duration* is thus a valid tool to elicit long-term habitual dietary intake pattern of subjects which could possibly be useful for the estimation of their nutrient intake in chronic diseases irrespective of the season of the year. As the RFQnFFQ used in this study is raw food based it could be adopted to other parts including rural areas of the country with appropriate modifications.

### **3.7 Carboxymethyl lysine induces EMT in podocytes and proteinuria in diabetes**

Advanced glycation end-products (AGEs) are implicated in the pathogenesis of diabetic nephropathy (DN). Carboxymethyl-lysine (CML) is one of the predominant AGEs that accumulate in all renal compartments of diabetic patients. The induction of the transcription factor Zeb2 in podocytes was demonstrated upon exposure to CML through activation of NF- $\kappa$ B signaling cascade. Zeb2 orchestrates epithelial-mesenchymal transformation (EMT), during which cell-cell and cell-extracellular matrix interactions enable epithelial cells to become invasive. While the exposure of podocytes to CML results in increased podocyte permeability, shRNA-mediated knockdown of Zeb2 expression abrogated CML-mediated podocyte permeability. Further, *in vivo* findings of elevated CML levels concurrent with increased expression of ZEB2 in glomeruli and proteinuria in diabetic rats confirm that CML-mediated manifestations in the kidney under chronic diabetes conditions. These *in vitro* and *in vivo* results envisage the novel axis of NF $\kappa$ B-ZEB2 in podocytes playing a significant role in eliciting EMT and pathogenesis of DN.

### **3.8 WT1-positive mesenchymal cells in pulmonary fibrosis**

Collagen-producing myofibroblast transdifferentiation is considered a crucial determinant in the formation of scar tissue in the lungs of patients with idiopathic pulmonary fibrosis (IPF). Multiple resident pulmonary cell types and bone marrow-derived fibrocytes have been implicated as contributor to fibrotic lesions because of the transdifferentiation potential of these cells into myofibroblasts. We demonstrate that Wilms tumor 1 (WT1), a known marker of mesothelial cells, is expressed by both mesothelial and mesenchymal cells in IPF lungs but has limited or no expression in normal human lungs. We also demonstrate that WT1+ cells accumulate in fibrotic lung lesions, using two different mouse models of pulmonary fibrosis and WT1 promoter-driven fluorescent reporter mice. Importantly, the number of WT1+ cells in fibrotic lesions was correlated with severity of lung disease as assessed by changes in lung function, histology, and hydroxyproline levels in mice. Finally, inhibition of WT1 expression was sufficient to attenuate collagen and other extracellular matrix gene production by mesenchymal cells from both murine and human fibrotic lungs. Thus, the results of this study demonstrate a novel association between fibrocyte-driven WT1+ cell accumulation and severe fibrotic lung disease.

### **3.9 Small heat shock proteins in rat heart under chronic hyperglycemia**

The induction of small heat shock proteins (sHsp) is observed under various stress conditions to protect the cells and organisms from adverse events including diabetes. Diabetic cardiomyopathy is a common complication of diabetes. Therefore, we investigated the expression of sHsp under chronic hyperglycemic conditions in rat heart. While the expression of MKBPHspB2, HspB3,  $\alpha$ B-crystallin ( $\alpha$ BC) was found to be increased in diabetic heart, expression of Hsp20 was decreased. Chronic hyperglycemia further induced phosphorylation of  $\alpha$ BC at S59, S45, Hsp27 at S82,

p38MAPK and p44/42MAPK. However, pS59- $\alpha$ BC and pS82-Hsp27 were translocated from cytosolic fraction to cytoskeletal fraction under hyperglycemic conditions. Furthermore, the results suggest up regulation of sHsp (MKBP, HspB3 and  $\alpha$ BC), phosphorylation and translocation of Hsp27 and  $\alpha$ BC to striated sarcomeres and impaired interaction of  $\alpha$ BC and pS59- $\alpha$ BC with Bax under chronic hyperglycemia.

### **3.10 Assessment of subclinical micronutrients status and non-communicable diseases of urban geriatric population: A population based cross-sectional study (Pilot study).**

In this cross sectional pilot study, the prevalence of subclinical micronutrient deficiencies, non-communicable diseases (NCDs) and their association in urban elderly people were reported. The prevalence of vitamin B12 and folic acid among urban elderly people was 36% and 8.2% respectively. The prevalence of vitamin B12 deficiency was significantly ( $p < 0.006$ ) high in males. The prevalence of vitamin D deficiency among elderly people was high (56.3%) and there was no vitamin A deficiency, whereas the prevalence of zinc deficiency was only 17.1%. The prevalence of diabetes, hypertension (HT), overweight and obesity in these subjects were 51.9%, 67.8%, 46.2% and 31.6% respectively. The prevalence of under nutrition was very low i.e., 2.1%. The prevalence of central obesity was 61.8% which was significantly ( $p < 0.002$ ) high in males when compared to females. The prevalence of dyslipidemia was very high (76.8%) and this was significantly ( $p < 0.004$ ) high in female subjects when compared to males. The prevalence of metabolic syndrome (MS) and cataract in these elderly subjects was 51.1% and 36% respectively. There was significant ( $p < 0.05$ ) association of vitamin D deficiency with HT, BMI, MS and also a significant ( $p < 0.05$ ) association of Zn deficiency with HT.

### **3.11 Effect of long-term pre-diabetes on risk of renal, retinal and lens abnormalities: Biochemical mechanisms and role of dietary agents.**

Two animal models were developed to study long-term pre-diabetes induced complications (retinopathy, nephropathy and cataract). Injection of streptozotocin (90mg/kg body weight) to two-day old Sprague Dawley (SD) rat pups (nSTZ) develops only impaired glucose tolerance (IGT) associated pre-diabetes by two months and maintains pre-diabetic state upto ten months. Feeding High fructose (HF), High fructose+ High fat (HFHF) to 45 to 60 days old WNIN rats resulted in IGT and insulin resistance associated pre-diabetes by three months. Both these models developed retinal and renal abnormalities by the end of ten months, but not cataract. However, when cultured these pre-diabetic rat lenses in high glucose medium for a period of four days develop early lens opacification when compared to their respective control group lens. Feeding of bitter melon (5.0%) to nSTZ pre-diabetic SD rats and feeding of garlic (3.0%) to HF fed WNIN rats had shown marginal protective effect in delaying development of these complications. Protective effect of bitter melon in nSTZ induced complications is mainly due to its mild hypoglycemic, aldose reductase inhibition and antioxidant properties. Protective effect of garlic in HF induced abnormalities is mainly due to its insulin sensitizing and antiglycating properties.

### **3.12 Vitamin A metabolism in relation to sexual dimorphism of adipose tissue development**

Male mice are susceptible to high fat-induced hyperglycemia, which could be partly explained by the RBP4 elevation in circulation, due to its over-expression, particularly in visceral adipose depots. However, no sexual dimorphic adipose tissue development between sexes, but triglyceride accumulation/storage in adipose tissues follows sexual-dimorphism; i.e. female accumulates more in gonadal depot and male accumulation in subcutaneous depot. Further, long term feeding of high fat diet increases hepatic vitamin A stores in both sexes of mice. However, most of the vitamin A metabolic pathway genes are transcriptionally regulated neither by sex nor by diet. Interestingly,

long term feeding of HF diet resulted in elevation of n-3 PUFA; docosahexaenoic acid (DHA; C22:6) levels of liver, possibly through ELOVL2-mediated chain elongation pathway, which may partly explain the amelioration of hepatic triglyceride accumulation. The present study also highlights the role of genetic-nutrient interactions/ relationships and its impact in determining the disease development and/or its progression, which implies the need for genetics-based intervention strategies for treating obesity and its associated complications particularly; insulin resistance and type 2 diabetes.

### **3.13 Vitamin A metabolism: a neglected paradigm in non-alcoholic fatty liver disease?**

Findings of the present study suggest that vitamin A deficiency induces hypotriglyceridemia and attenuates high fructose-induced hepatic steatosis by regulating key factors involved in triglyceride biosynthesis, such as glycerol 3-phosphate dehydrogenase (GPDH) and stearoyl CoA desaturase 1 (SCD1). In addition, increase in docosahexaenoic acid (DHA; C22:6) and its active metabolite resolvin D1 (RvD1) levels, implicates their significant contributions to the vitamin A deficiency-mediated favorable changes in hepatic lipid metabolism. Notably, chronic high fructose feeding, though resulted in hepatic steatosis, it did not affect the retinol status and its metabolic pathway genes/ proteins expression in liver. However, the key adipocytokine; leptin levels was markedly reduced by vitamin A deficiency diet feeding, which corroborates with decreased adiposity observed in these groups. Further, the data showed improved insulin sensitivity and glucose clearance, due to chronic vitamin A deficiency diet feeding. Overall, the study underscores the importance of nutrient-nutrient interaction in determining health and disease conditions, which assumes greater significance, in view of the therapeutic potential of vitamin A and its metabolites in clinical research.

## **4. EXTENSION AND TRAINING**

### **4.1 Influence of mass media on teenagers' diet and health-related behaviour**

This cross sectional study explored the influence of mass media on adolescents' diet and health-related behaviour (HRB). The objectives were to assess media viewing habits of teenagers; to assess the media content which the teenagers' are exposed to; to assess teenagers' understanding and adoption of media content on diet and HRB; to study teenagers' perceptions and practices of diet and HRB; and to elicit influence of mass media, if any, on diet and health of teenagers. In all 517 (253 boys 264 girls) participated in the study from 21 schools/Junior colleges of greater Hyderabad. The results highlight variation between same age group children and between genders in usage of media and understanding of media messages. Mass media influence on teenagers varied broadly based on their families' economic status. Among each group again influence of media was divergent between genders. The study reveals the influence of mass media on teenagers' consumption of alcohol and tobacco products. A sum of 24% teenagers is influenced by mass media towards this unhealthy behaviour. Teenagers used these products to imitate media visuals, film actors, television advertisements/ celebrities. Comparatively, boys of middle-income families are more under influence of film stars and consume alcohol and tobacco products to imitate actions on the screen. More than one-third of teenagers of this study do not do any physical activity. The primary reasons they mention for physical inactivity are "homework pressure", "lack of play ground" "watching television" or "Internet browsing". Majority of Adolescents pester their parents to purchase food and beverages endorsed by celebrities.

## 5. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

### 5.1 Role of tamarind extract on the carbonic anhydrase activity in ameliorating fluoride toxicity in rats

Fluoride administration inhibits carbonic anhydrase activity of RBC and in kidney homogenate in fluoride intoxicated rats compared to control rats. Tamarind fruit extract (TFE) supplementation to fluoride intoxicated rats enhanced carbonic anhydrase activity with increased urinary fluoride excretion. Long term exposure of Fluoride showed a trend of impaired glucose tolerance in rats.

#### Integrated fluorosis mitigation activities in Nalgonda District

In Nalgonda district, Out of 2066 students screened for dental fluorosis in school children, 1183 students (57.2%) were affected by dental fluorosis. The percentage prevalence of fluorosis in each category villages was 16% (Category I; water fluoride level 0.83 ppm), 47% (Category II; water fluoride level 2.06 ppm), 81% (Category III; water fluoride level 2.83ppm) and 82% (Category IV; water fluoride level 3.8 ppm). Oxidative stress studies revealed the increased oxidative damage in High ODAP treated group compared to the control group. Histopathological changes indicated extensive degeneration of motor neurons in high ODAP treated group compared to the low ODAP treated group.

### 5.2 Studies on *salmonella* decontamination of foods using hybrid technology of ozone-pulsed UV

Contamination of spices with *Salmonella* is an important cause of detention and rejection of shipments of spices in export markets. Thermal methods of pasteurization or sterilization cannot be used for spices to remove this pathogen. In recent years ozone gas has been recognized as very powerful antimicrobial agent and can be used where thermal methods are not useful. The study on effect of ozone in combination with UV in reducing *Salmonella* and its contamination in spices demonstrated that ozone in combination with UV was effective against *Salmonella* decontamination. Combination of ozone and UV caused lethality that was greater than the sum of lethality of ozone applied individually. The study showed that 0.2 ppm of ozone was effective against *Salmonella* Spp. than 0.8 and 1.4 ppm. There was 100% inactivation of *Salmonella* with the usage of ozone and UV at 0.2 ppm at 20 min of exposure time. About 20 min of exposure time was more effective in reducing the growth of *Salmonella* when compared to 5, 10 and 15 min of exposure time. A significant reduction of *Salmonella* in pepper (98.76%) was observed at 1.4 ppm concentration of ozone and 15 min of exposure time along with 30mins of UV exposure. Inactivation of *Salmonella* spp. (100%) in ground pepper (n=40) was observed at 1.4ppm conc. of ozone and 15 min of exposure time along with 30mins of UV exposure. A significant reduction of *Salmonella* (85.9%) in chilli was observed at 1.4 ppm conc. of ozone and 15 min of exposure time along with 30mins of UV exposure. Ozone in combination with UV can be an effective treatment for reduction in *Salmonella* contamination of spices such as chilli and pepper. The results demonstrated that the hybrid technology of ozone pulsed UV is a promising alternative technique for *Salmonella* decontamination in spices.

### 5.3 Emerging bacterial foodborne pathogens in milk products

A study was conducted to determine the prevalence of emerging foodborne pathogens in milk products. The study on emerging bacterial foodborne pathogens in milk products indicated the presence of foodborne pathogens like *Salmonella* and *S.aureus*. The other indicator organisms like *E. coli* and fecal coliforms were also detected in the milk products. The contamination of *S.aureus* (73.5%) was high in khoa than other milk products. The other emerging foodborne pathogens like *Listeria* Spp. *Methicillin resistant staphylococcus aureus* (MRSA), *Yersinia enterocolitica* and *E.coli*

*O157:H7* were not detected in any of the milk products. Khoa (52%) samples were found to contain *S.aureus* above  $10^6$  cfu/g which is likely to produce heat stable enterotoxin. Among 143 (31.7%) cultures of *Staphylococci*, 106 (74.1%) showed coagulase enzyme production and 37 (25.9%) isolates were coagulase negative. Only nine cultures (6.3%) showed positive result for enterotoxin production. It is known that  $>10^6$  cfu/g of *S. aureus* is likely to produce enterotoxin, however in the present study 17% of food samples have crossed the limit but very less number of them were able to produce enterotoxin. There is a need to carry out a detailed study on evaluation of coagulase production among *Staphylococci* and its enterotoxin production. The presence of *Salmonella* Spp. in milk product is a cause of concern from the consumer point of view. The counts of *Salmonella* and *S.aureus* are not conforming to FSSAI-Microbiological standards of foods.

#### **5.4 Safety and quality of rice and wheat distributed in PDS with special reference to damaged grains and mycotoxin contamination**

A project on Safety and quality of rice and wheat distributed in PDS with special reference to damaged grains and mycotoxin contamination was initiated in 2011 to assess quality and safety of rice and wheat during their distribution in the PDS chain. The main objective was to evaluate fungal and mycotoxin contamination in rice and wheat stored under government storage units and PDS centres and to assess the mycotoxin levels in damaged grains segregated from food grains distributed in PDS. Analysis of 24 samples consisting of raw milled rice, boiled rice and wheat collected from storage godowns in Cherlapalli, RR district, Hyderabad indicated that levels of aflatoxin were below the FSSAI/GOI tolerance limit of 30µg/kg in all the analysed samples. Aflatoxins were also not detected above the regulatory levels in 9 PDS samples collected from households and 25 samples non-PDS rice samples collected from the retail markets. In rice products such as broken rice, rice rawa, rice flakes and rice flour samples presence of aflatoxins was detected in 10/37 broken rice but at levels below the FSSAI limits (1.0- 14.3g/kg). Aflatoxin levels in damaged grains segregated from rice samples indicated presence of fully damaged/dicoloured grains (0.03-0.4%), partially discoloured grains (0.1-0.8%) and dull looking grains (0.3-1.7%) in which aflatoxins were detected at levels of 0.1, 0.2, 3.0 and 4.0g/kg respectively. Presence of ergosterol assessed in 9 brown rice and 21 milled polished rice samples was found at levels ranging from 0.5-1.0µg/g and 0.25-14µg/g in brown and milled rice respectively. The above study indicated that aflatoxins are not present at levels above the food safety limits in PDS samples. The study observed that a potential for occurrence of higher aflatoxin may exist due to presence of damaged grains.

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

The project on Assessment of allergenicity potential of novel proteins expressed in genetically modified (GM) plants under varying conditions of digestion and thermal treatments was initiated in November 2009 with the objective of testing purified novel proteins expressed in GM crops for their stability to pepsin digestion and heat. The assay developed consisted of 3 components namely determination of limit of detection of the novel protein in order to measure 90% digestibility of the test protein in simulated gastric fluid with pepsin at pH 1.2 on SDS-PAGE, determination of pepsin activity so that the required activity level of enzyme to protein is maintained at 10:1 during digestion of test protein, and digestion of test protein in SGF at pH 1.2. The method was validated with various purified proteins that are known to be stable or unstable to pepsin digestion ( $\beta$ -lactoglobulin, ovalbumin, concanavalin A, bovine serum albumin, lysozyme, RUBISCO) and applied to testing of 6 novel proteins expressed in GM crops namely Cry1Ac and Cry1EC, (BT cotton),

Cry1Fa1 (BT brinjal), Bar, Barnase and Barstar recombinant proteins expressed in GM mustard, for stability to pepsin digestion as part of pre-market regulatory approval. All the novel proteins expressed in GM crops tested have been shown to be rapidly digested within 0.5 minutes thus indicating limited risk of food allergy for these proteins. The effect of varying pH of SGF and pepsin activity levels on extent of digestion was evaluated using purified proteins which showed that pepsin is active upto pH 3.5 in SGF and beyond that becomes inactive and hence cannot digest the proteins. The stability of novel proteins to varying temperatures was tested at 0-95°C for insecticidal proteins namely Cry1Ac, Cry1EC, and Cry1Fa1 and enzymatic proteins namely Bar, Barnase, Barstar. The activity of the heat treated novel proteins tested using insect bioassays and enzyme activity assays showed that all the proteins were rapidly inactivated by heat at temperatures tested. Through the above project, the pepsin digestibility assay and thermal stability assay could be successfully established for testing GM crops and regulatory reports submitted to the RCGM GOI for approval.

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

### **6.1 Paternal diet restriction and metabolic gene expression studies in obese rat offspring**

Studies have suggests that paternal diet along with maternal diet has concurrent effect in programming the offspring to various metabolic complications such as obesity, type 2 diabetes and other associated disorders. In this study the effect of paternal diet restriction on modulation of various metabolic pathways involved in carbohydrate and lipid metabolism in the offspring studied. Upon diet restriction of WNIN/Ob obese male rats from different age group, the circulatory cholesterol and triglycerides, were reduced significantly when compared to the age matched *ad libitum* fed group. It was also noticed that the levels of TBARs and TOC were significantly reduced upon diet restriction. Hence, diet restriction in male obese WNIN/Ob rats lowered the rate of body weight gain, with reduced oxidative stress overall and fertility restoration in groups at early development stages of intervention. Food restriction reduces obesity but does not improve Leptin gene expression in WNIN/Obese rats. Further, it was observed that the obese pups born to diet restricted obese males have higher body weight gain, mean body weight and higher lipid profile compared to obese pups born to adlibitum fed carrier males.

### **6.1 Genetic and epigenetic approach towards obesogenesity using a rat model**

Experimental data suggested that the all 4 rat strains studied showed a differential response towards diet source. Experimental groups of WNIN showed altered glucose metabolism associated with defects in insulin sensitivity and insulin secretion as evidenced by higher plasma Insulin levels, scoring higher values for HOMA IR and HOMA-beta and lower values for Insulin Sensitivity Index (ISI). Further it was noticed that WNIN developed dyslipidemia condition in high calorie fed groups as evidenced by significant increase in abdominal fat, elevated levels of circulatory triglycerides and decreased HDL-cholesterol levels and showed significantly increased levels of inflammatory cytokines such as IL-6, MCP-1, IL-1beta, and TNF alpha. Visceral adipose tissue histology studies revealed that, increased hypertrophy and hyperplasia, and mean adipocyte area compared to controls in WNIN strain. However, in SD and F-344, there was no such phenomena was observed under high calorie environment. The transcriptome analysis of adipose & liver tissues of WNIN and Fischer-344 strains fed with high calorie diets (High fat, high fat Sucrose) showed differentially expressed genes and their specificity towards diet, species and organ.