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^+ and Bsh^- indigenous probiotic Lactobacillus plantarumstrains 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+ and Bsh- indigenous probiotic - Lactobacillus plantarum strains of human origin on cholesterol metabolism of rats. Bile salt hydrolase active (Bsh+) 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_{12} 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_{12} status and its association with leptin and anthropometric indices of adiposity among urban adolescent boys belonging to low and middle income groups. Vitamin B_{12} 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_{12} 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}Fe tracer and the conventional colorimetric methods. Both the isotopic and florescent 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 H2O2. 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 -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 -HSD1 as a key strategy to treat metabolic syndrome. This perhaps is the first study to link 11 -HSD1 to adipose tissue fibrosis and tissue glycogen content under obese condition. Feeding of diet rich in vitamin A decreased 11 -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 -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 glycations systems have demonstrated. While the antiglycating action of EA seems to involve predominantly inhibition of N -(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 inter-personal 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 byICMR. 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.

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 absorbtiometry (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

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 200microsatellite 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 immunologlobulins, 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 (turdal) 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.