RESEARCH HIGHLIGHTS

1. COMMUNITY STUDIES

1.1 Prevalence and determinants of Hypertension and Type 2 Diabetes among 20-60 years old urban dwellers in Hyderabad, Andhra Pradesh

In the hypertension and diabetes study, 3102 urban adults (women: 50.6%) of 20-60 years were covered. The majority of urban men and women were literate (85%) and half of the men and one third of the women were educated (college and above). The mean BMI (kg/m^2) of men and women of 20-35 year adults was 23.1 (±4.36) and 23.8 (±4.84) respectively, while it was 25.4 (±4.44) and 27.4 (±4.91) among 36-60 years.

As per the BMI values for Asian, the prevalence of overweight and obesity was 46.2% and 51.9% among men and women of 20-35 years respectively, while it was 70.2% and 83.5% among 30-60 years. The prevalence of abdominal obesity (waist circumference; men: ≥ 90cm; women: ≥ 80cm) was 28.8% and 37.7% among men and women of 20-35 years, respectively, while it was 58.8% and 69.6% among 36-60 years.

The prevalence of hypertension (SBP ≥ 140 mm of Hg and /or DBP ≥ 90 mm of Hg) was 40.6% and 30.4% among men and women of 20-60 years respectively, while it was 55.8% and 49.3% among 36-60 years. The prevalence of T2 diabetes (≥ 26mg/dl) was 3.6% each in men and women of 20-60 years, while it was 21.9% and 19.4% among 36-60 years. It was significantly higher among the adult men and women who had obesity/abdominal obesity compared to normals.

Only 5% men and 4% women rated their health as excellent. About 80% men and 60% women were aware of their body weight and one third of men and half of the women were also aware that they had excess body weight and more than half of them were trying to lose their weight. Only one fourth of men and 10% women were doing mild to moderate exercise daily. Almost all men and women were watching TV at least two hours daily.

1.2 Survey conducted on physical and mental disabilities reported in the "Payakarao Peta" Legislative Assembly constituency of Visakhapatnam district in Andhra Pradesh

The objectives of the survey was to investigate high prevalence of physical deformities reported in "Payakarao Peta" assembly constituency of Visakhapatnam district and to recommend necessary interventions.

The preliminary survey conducted by NIN scientists revealed that 41 out of 11,350 people from four villages were affected with unusual disabilities. The overall prevalence was around 0.35% as against the national figure of 2.1%. In general, the disabilities were, mental retardation, physical deformities such as talipes equinovarus, genuvarum, kyphosis, deaf-mutism and blindness, with most of the cases among children of 10 years and above. About 50% of them reported history of birth asphyxia due to inadequate availability/utilization of health care facilities and history of consanguinity.

The expert committee survey in 7 villages covering 2.2 lakhs population revealed the presence of 58 out of 108 cases with mental retardation with or without cerebral palsy. Other deformities observed were microcephaly, deaf-mutism, blindness due to retinitis pigmentosa and

micropthalmia, dwarfism, cleft palate, talipes equinovarus, brachydactily, floppy child syndrome, choreo-athetosis, post polio residual paralysis, down's syndrome, trauma and hemiplegia. The independent survey conducted by the State government also revealed a prevalence of about 0.7%, which is also in line with the expert committee findings and much lower than the national average.

It was observed the reported problem was not unique/ specific to the area, reuiring in depth research evaluation. It was recommended that problem needs to be attended through medical, psycho-social, financial support and occupational rehabilitation through a multidisciplinary plan, involving local NGOs and philanthropic organizations. There is also need to generate data on community disability through a well designed epidemiological fact finder to guide future planning.

2. MICROBIOLOGY AND IMMUNOLOGY

2.1 To evaluate the efficacy of a *lactobacilli* preparation on bacterial vaginosis and vaginal immunity in healthy subjects and in patients with BV

- The three strains of *lactobacilli* (*L.brevis, L.salivarius Salicinius, L.plantarum*) induced resolution of BV in 76.5% of the women and restored normal vaginal flora (NVF) in 80 % of the women after 8 days local treatment.
- Cervical erosion and leucorrhea (vaginal WBCs) resolved substantially with local *lactobacilli* treatment.
- However, the effect on vaginal flora was very transient. The efficacy reduced to 60% by 15 days suggesting a longer and repeat treatment.
- The pH measurement for diagnosing BV is a good tool in resource-poor settings, because although it is not the most sensitive or specific test, it offers a middle ground on sensitivity and specificity compared with the more technologically demanding techniques. Moreover, this pH test performs better than the syndromic diagnostic algorithm.
- In symptomatic women, a high vaginal pH result would require further evaluation by a health care provider.

2.2 Effect of HIV on growth, morbidity and disease progression in HIV Infected children

- CED was prevalent in 19.5% at baseline and 23.9% at 1 yr.
- Undernourishment (38%), Stunting (45.5%) were quite prevalent.
- Nearly 50% were anemic, and they suffered from Iron, Folic acid and Vitamin D deficiency.
- In children receiving ART: Heights and weights increased significantly, Fat % and LBM were better and HIV viral load was lesser. Furthermore CD4 and CD8 counts were better.
- In children with morbidity – CD4 and Cd8 counts were lower and viral loads were higher.
- Serum albumin, Copper, Vit B_{12} correlated positively with CD4,CD8.
- Albumin correlated negatively with Viral load.
- Vitamin B_{12} correlated negatively with Ratio.

3. CLINICAL STUDIES

3.1 Calcium-rich food supplementation to lactating women from low socio-conomic group- Effect on bone density

Gingelly (til) a calcium-rich food when supplementated to under nourished women from the low income group resulted in significantly reduced loss of bone mineral density at the femoral neck and hip regions when compared to the control group. The study advances the knowledge in the area of bone metabolism during lactation and provides important information for the calcium requirements of lactating women.

3.2 Case control study of osteoporotic hip fractures

- Vitamin D levels were significantly (P< 0.001) lower in the fractures cases than the controls.
- There was high urinary fluoride excretion in the fractured cases and significantly (< P 0.001) more than the controls.
- At the femoral neck, 51% males and 76% females had osteoporosis, while, in the controls 19% males and 41% females had osteoporosis.

4. BASIC STUDIES

4.1 Establishment of screening facility for iron and zinc bioavailability using Caco2 cell- line

Biofortification of staple food crops such as rice, wheat and maize for iron, zinc and beta carotene through conventional plant breeding is considered as a sustainable strategy for improving their nutritional status in the population. During the process of development of a bio fortified product, screening for bioavailability of micronutrients assumes importance to ensure the intended beneficial outcome of these strategies. As a coordinating center for the nutritional studies of the Crop biofortification network project, a human intestinal cell line model, Caco-2 cell line, based bioavailability screening facility was established at this institute. Bioavailability screening methods for iron, zinc and carotenoids were developed and validated in the facility. The screening method for assessing the zinc bioavailability was used in selecting genotypes from 4 quality protein maize (QPM) variety and 6 hybrid maize genotypes with varying zinc content from 0.76- 2.34 mg/100g. Among the maize genotypes tested, 2 hybrid maize varieties showed the highest zinc content and bioavailability. Thus, the bioavailability screening method using Caco2 cell line can form an essential step in selecting genotypes for crop biofortification.

4.2 Development of a valid and reliable questionnaire for testing knowledge on micronutrients among adolescent students

Micronutrient deficiencies are rampant in India among all age groups. Absence of culture-appropriate nutrition education has been identified as one of the weakest links of nutrition intervention programs in India for targeting micronutrient malnutrition. One of the obstacles could be the lack of information on current nutrition knowledge of different communities, which in turn is attributable to the lack of validated questionnaire for testing knowledge among target groups. Since adolescence is an age group where maximum behaviour change is possible, the aim of the present study was to develop a psychometrically valid and reliable questionnaire for testing knowledge on micronutrients and to apply the test among a group of adolescents (16-18y) where micronutrient status was analyzed and to assess the relationship between knowledge and the biomarkers of micronutrient status. An 18 item questionnaire for adolescents was constructed with acceptable

validity and reliability. Knowledge on micronutrients measured using this questionnaire was found to be a significant predictor of plasma retinol status. This emphasizes the need for using validated questionnaires in nutrition research involving knowledge and if it is assessed correctly will relate to the nutritional status of the individual.

4.3 Maternal vitamin B12 and/or folate restriction induced changes in body adiposity, hyperglycemia and insulin resistance in Wistar rat offspring: molecular basis of the changes

In the earlier report, a Wistar rat model with chronic dietary restriction of folate and / or vitamin B_{12} increased body fat % (visceral adiposity) and fasting plasma glucose but not their insulin resistance status. While chronic maternal folate and / or vitamin B_{12} deficiencies increased the body weight of the offspring at/from weaning, altered their body composition (visceral adiposity) and induced insulin resistance (fasting and postprandial), most changes were mitigated at least partly by rehabilitation indicating their probable reversibility. Overall, chronic maternal folate and/ or B_{12} deficiency appeared to predispose the offspring to insulin resistance. Considering that abundant literature suggests trans-generational transfer of the effects of maternal under-nutrition in the offspring, we assessed whether or not the maternal folate and / or vitamin B_{12} restriction induced changes are transmitted to F2 generation. It was interesting to note that maternal folate and / or vitamin B_{12} restriction induced body composition changes were also seen in the F2 offspring. However, it was perplexing to see that changes were observed only in the male but not female offspring, both in F1 and F2 generations. The reasons for this gender bias are to be investigated.

4.4 Insulin, Insulin receptor and its signaling mechanisms in the brain and insulin sensitive target organs in NIN obese mutant rats (WNIN/Ob and WNIN/Gr-Ob) and Central regulatory mechanisms underlying obesity in WNIN Obese mutant rats

WNIN – Obese (sumo) rat developed at NCLAS, NIN, Hyderabad, India resemble Neuron specific insulin receptor knockout mice in that they are both hyperphagic, hyperinsulinemic, obese and infertile. These studies were conducted in six months old female WNIN/Ob rats to validate / negate the hypothesis, “impaired brain / hypothalamic insulin function / signaling could underlie the hyperphagia, obesity, in WNIN/Ob rats”. The findings suggest that impairment in brain / hypothalamic insulin signaling along with the impaired hypothalamic glucose uptake/energy homeostasis and the resultant failure to attain satiety in addition to decreased neuropeptide receptors and serotonin metabolism could either singly or together be responsible for the hyperphagia and obesity observed in the six months old female in WNIN/Ob rats.

4.5 Effect of different methods of cooking on natural antioxidant activity and phenolic content of green leafy vegetables commonly consumed in India.

Continuing the efforts to generate a database on phenolic content and antioxidant activity of plant foods commonly consumed in India including the effects of common domestic processing, this year the effect of conventional, pressure and microwave cooking on these parameters in green leafy vegetables was studied. In general, nine out of the eleven GLVs studies showed significant increase in their phenolic content and antioxidant activity on different types of domestic processing while only two them showed decrease. The fact that there was significant rank correlation among the PC and AOA among all GLVs studied both raw and processed suggests the importance of phenolics to the AOA of the GLVs studied in both these forms.

4.6 Vitamin B_{12} deficiency and hyperhomocysteinaemia in diabetic retinopathy

Although, many studies indicated an association between homocysteine and diabetic retinopathy (DR), the results so far have been equivocal. Amongst the many determinants of

homocysteine, B-vitamin status was shown to be a major confounding factor, yet very little is known about their relationship in DR. In the present study we found that higher homocysteine levels in DR were associated with lower vitamin-B_{12} but not with other B-vitamins. Additionally, hyperhomocysteineamia and vitamin-B_{12} deficiency do not seem to be related to the age, BMI and duration of diabetes. These results thus suggest a possible association between deficiency of plasma vitamin-B_{12}, hyperhomocysteinaemia and DR, for the first time. Further, these studies also indicate that vitamin B_{12} deficiency could be an independent risk factor for DR.

4.7 Animal model for type-2 diabetic complications

Chronic diabetes leads to various secondary complications. Although, there are many studies on complications of diabetes in experimental animals, most of the studies are conducted on type-1 diabetic animal models. However, barring some genetic models, there are no studies on type-2 diabetes (T2D)-induced complications in experimental conditions. A series of animal experiments were conducted with various T2D rodent models to evaluate a suitable animal model of T2D not only to understand the possible mechanisms involved in the development but also to prevent or delay diabetic complications. These studies indicate that neonatal-streptozotocin (nSTZ) WNIN-GR/Ob model could serve as a suitable model for studies on T2D-induced complications, particularly diabetic cataract and also for dietary intervention studies.

4.8 Novel ALR2 inhibitors

Aldose reductase (ALR2) catalyzed accumulation of osmotically active sorbitol has been implicated in the development of diabetic complications. A new natural active principle (piplartine) has been isolated from black pepper with ALR2 inhibitory potential, and using this natural molecule as a lead molecule, a novel hybrid compounds as ALR2 inhibitors by chemical transformation through Michael addition were synthesized. These novel compounds (particularly 3c, 3d, 2j and 3e) are more potent than the well know inhibitors sorbinil and fidarestat. Thus, these novel ALR2 inhibitors might be useful for the treatment and/ or prevention of diabetic complications.

4.9 Importance of α-crystallin heteropolymer

Eye lens α-crystallin exists as a heteropolymer composed of two homologous subunits, αA and αB. Despite the critical role of α-crystallin in many tissues, little is known regarding structural and functional significance of the two subunits. Herein, we describe a unique feature of αB-crystallin. At high temperatures not only αB-crystallin aggregates but also enhances the aggregation of other lens proteins. Intriguingly, αB-crystallin-mediated coaggregation involves β - but not γ-crystallin Further, α*A*-crystallin, but not a mutant (F71L) α*A*-crystallin, prevented aggregation of αB-crystallin and also reduced coaggregation of αB- and β-crystallin. These studies explain the rationale for the existence of α-crystallin heteropolymer with αA subunit as a major partner that is vital for lens transparency. Hence, heteropolymer with 3:1 αA to αB ratio might be vital for eye lens transparency under diverse conditions to prevent cataract.

5. FOOD AND DRUG TOXICOLOGY

5.1 Flagship Project WNIN/Ob mutant rat model to studyDNA damage and mutagenicity testing

NCLAS at NIN has established two obese mutant rat models- WNIN/Ob and GR/Ob, former with euglycemia and the later with hyperglycemia. These animals show distinct physical, physiological and biochemical indices of obesity and age faster than the normal wistar rats. Apart from obesity these rats shows incidence of tumors (60%), cataract (10%), opportunistic infections (100%) and kidney abnormalities (80%). Carcinogenic process is known to be preceded by

damage to DNA. This is known to induce alterations in cellular genome and altered gene expression. Accumulations of such mutations are associated with ageing and other mutation based degenerative diseases like cancer, diabetes, cataract etc. Since these mutant rats shows obesity and obesity related degenerative chronic disorders, it is possible that they harbour large proportion of damaged DNA & accumulation of age related end products.

The result of the study showed that there was no significance in the antioxidant status of DNA damage and antioxidant enzymes in obese and lean rats compared to the WNIN rats as they did not exhibit any induction in the strand breaks in the blood tested as evidenced by the alkaline comet assay.

5.2 Assessment of Environmental Lead Exposure on Infection and Immunity

It is known that Lead, the ubiquitous environmental pollutant causes sub-clinical organ system damage specially to haemopoietic, renal and nervous system. Undernutrition *per se* may aggravate lead toxicity. Current evidences suggest that elevated lead levels alter immune functions by enhancing lymphocyte proliferation and possibly increase severity of infectious diseases. Micronutrient deficiency specially Fe may hamper immune function.

Elevated levels of lead and micronutrient deficiency may alters immunity and enhance lead induced cytotoxicity. Against this background a study was taken up with the following objectives,

1. To assess the immune function in Pb exposed iron deficient animal model.
2. To determine the effect of oral Pb exposure on intestinal microflora in iron deficient rats.
3. To evaluate the protective effects of thiamine on Pb induced inhibition of *Lactobacill* and *E. coli*.

The results revealed that chronic Pb exposure even at low levels can reduce the immune functions in iron deficiency. The *in vitro* bacterial culture studies have shown protective effect of thiamine against Pb induced bacterial inhibition.

6. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

6.1 Effect of Mucuna pruriens on WNIN obese rats

In Indian system of medicine, several medicinal plant extractions are used to prevent or to treat human diseases. The ancient Unani medicine, mentions the use of *Mucuna pruriens* mainly as an aphrodisiac. We tried to ascertain the ascribed medicinal properties of *M.pruriens* ethanolic seed (velvet beans) extract in an unique inbred obese mutant rat model – WNIN/GR-Ob, an obese rat with prediabetes established at our institute. The obese male and its lean counterparts were fed with 0.5 gm hand made pellet containing 6 mg of the seed extract (normal dose group), and 1.0gm hand made pellet containing 12 mg of the seed extract (high dose group) for 45 days. The rats were analyzed for lean body mass (LBM), body fat and extra cellular fluids by a Total Body Electrical Conductivity (TOBEC) instrument. The circulatory levels of plasma glucose, cholesterol, triglycerides, reproductive hormones like testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin were also measured. Levator ani (LA) muscle weight and semen analysis parameters like sperm motility, sperm count, gonadial index and histology of testis were investigated additionally, along with histology of liver and testis.

The experimental rats showed a significant decrease in body fat, blood glucose and lipids, with an increase in LBM, total body sodium and potassium and fat free mass as compared to controls. Serum testosterone, prolactin, LH and FSH hormone levels showed a significant increase in treated rats as compared to controls. Among the treated groups the high dose treated animals

showed significantly higher values compared to normal dose treated rats. The LA muscle weight was increased 2-3 times and also a significant increase in terms of sperm count and motility was found in extraction treated rats compared to control rats. Histologically, steatosis of fat was significantly reduced in the livers and significant improvement was seen in the architecture of the testes with densely packed spermatids in the seminiferous tubules of treated obese rats. Thus, the seed extract of *M.pruriens* was found to have significant benefits in terms of reduction in total fat content, lipids and increase in lean body mass as expected and additionally in hypoglycemic effect as well. It also showed its potential as an aphrodisiac in terms of improvement of reproductive hormone profile and semen quality in infertile WNIN obese rats. The hypolipidemic effect shown in this study, is a new finding which adds to the list of other benefits of this traditionally used aphrodisiac.