NUTRITION

The Council’s National Institute of Nutrition (NIN), Food and Drug Toxicology Research Centre and National Centre for Laboratory Animal Sciences at Hyderabad continued to lay emphasis on providing fresh insights into nutrition research and streamlined both basic and applied research to make it more need-based, accountable and cost-effective. In addition, open ended research was also supported by the Council. Assessment of diet and nutritional status was carried out by National Nutrition Monitoring Bureau (NNMB) in nine States during the year. In addition, 17 districts of West Bengal were covered to determine district level nutrition profile. Research studies showed a higher prevalence of overweight and obesity among the adolescent school-going children belonging to high income groups. The effect of blood lead level on the cognitive performance of children and the suitability of human development index (HDI) in defining health and nutritional status were also studied. Studies on infant feeding practices revealed the need for appropriate nutrition education for mothers on aspects of supplementary feeding.

COMMUNITY STUDIES

Diet and Nutrition Survey in Rural Areas

During the year under review collection of 2000-2001 data on food and nutrient intake from 30,968 individuals from 7,131 households was completed in nine states. The results were compared with a similar survey carried out in 1990-91. The proportion of individuals in any age group, receiving 100% of recommended dietary allowance (RDA) for all the nutrients was less than one per cent. However, with respect to energy and protein, about a third of men and non-pregnant non-lactating (NPNL) women consumed 100% RDA as compared to 7-14% children. At both the points, the dietary intakes were essentially similar and the intakes of vitamin A and riboflavin were much below the RDA. Significant reduction was seen in the prevalence of severe grade undernutrition from about 30% in 1990-91 to 21% in the present survey (Fig.1).

![Fig.1. Distribution (%) of pre-school children according to SD classification](image-url)
Trends in Breast Feeding Status of Rural Infants

Data was collected by NNMB on infant feeding practices during the year 2000. Comparison with 1990-91 data showed that breast-feeding continued to be universal at both points of time but the proportion of children breastfed upto three months of age showed a significant improvement in the year 2000. More than 90% children of >4 months of age were solely breast fed at both time points. A large proportion of mothers initiated complementary feeding to their infants by about 6 months of age. Though these results are encouraging, it is important that the rural mothers are educated about the type and the quantity of complementary feeds to be given to infants.

Development of Low Cost Nutrient Supplement for Malnourished Children

Studies carried out so far have indicated that the three recipes developed by Central Food Technological Research Institute (CFTRI), mainly Suruchi Meetha, Nutro Crispo Sweet and Salt and NDDB’s chikki (Fig.2) were stable and accepted both by children and adults. The quality of the protein and the growth promoting effects in rats were acceptable. The amino acid composition is also of good quality.

Fig.2. Nutritive recipes developed by CFTRI.

Nutrition Profile of Indians – A District Level Survey in West Bengal

Assessment of nutrition profile in 17 districts of West Bengal indicated that the extent of undernutrition in almost all the districts was more than 50% except in the districts of Kolkata (46.4%), Purulia (47.9%) and Midnapore (48.6%). The diet and nutrient intakes especially micronutrients were inadequate compared to RDI. The data will be useful for planning at district level.
Cognitive and Neuropsychological Effects of Long-term Lead Toxicity

Blood lead levels exceeding the safe limit of 10 µg/dl have been shown to be associated with lowered performance on tests of intellectual ability. Study undertaken at NIN to assess cognitive and neuropsychological functions in children involved in bangle making in four urban slums in Hyderabad showed that not only the mean blood lead level in these children was high (17.3 µg/dl) but children in lead handling occupations had significantly high (≥25µg/dl) blood lead levels. None of the cognitive variables were related significantly to either increasing or decreasing levels of lead. However, significantly higher percentage of children involved in lead handling occupations had poor neuropsychological status.

Integrated Child Development Services in Andhra Pradesh

The results of a baseline survey of World Bank sponsored integrated child development services (ICDS) under Andhra Pradesh Economic Restructuring (APER) programme indicated that more than three-fourth of the target population was availing ICDS services. The proportion of 6-35 month old children participating in Supplementary Nutrition Programme (SNP) was relatively less than the older children. Only about 21-27% younger children consumed the supplement at the centre itself. The performance with respect to distribution of iron and folic acid (IFA) tablets and vitamin A was not satisfactory. Growth monitoring was also not regular. However, the coverage under immunization was satisfactory. These results indicate the need for strengthening the nutrition and related services under ICDS.

Human Development Index: Suitability for the Assessment of Health and Nutritional Status of Indian States

Human Development Index (HDI) is being widely used for measuring the health inequality and standards of living in various countries. Suitability of this index in explaining the health and nutritional status of 22 Indian states was assessed. The HDI was higher for Goa, Delhi, Maharashtra, Kerala and Punjab (known to be good performers in the field of health and nutrition) and lower for Bihar, Madhya Pradesh, Uttar Pradesh, Orissa, Assam and Rajasthan, (poor performers in this field). Further analysis showed that Kerala with higher HDI had better educational status of both sexes, low mortality rate in all ages, better nutritional status, better health indices and higher physical quality of life. These results indicate that education and women’s empowerment can improve the health and nutritional status of the community.
Effect of Seasonality on Birth Weight of Infants

The birth weight of a newborn is a sensitive indicator of the nutritional and health status of the mother and child. It is presumed that seasonality affects the intake of micronutrient rich food by mothers which in turn affects the birth weight of infants. To confirm this, 1989 data of about 13000 births and 1998 data of 16000 births in Safdarjang Hospital, New Delhi were analysed by Nutrition Foundation of India, New Delhi. Monthly segregation of data indicated variation in the mean birth weight of newborns during different time periods. In both the years, the infants born in March had the highest mean birth weight while those born in August had the lowest (Fig.3). The number of low birth weight deliveries was minimum in March (22-24%) and maximum in August (32-36%) (Fig.4).

Fig.3. Seasonal differences in birth weight observed in 1989 and 1998 in Safdarjang Hospital
Nutritional Status of Affluent School Going Children in Delhi

Anthropometry has become the conventional practical tool for evaluating the nutritional profile of populations. So far, National Centre for Health Statistics (USA) reference values have been used, but these are not applicable in developing countries. A study was conducted by Nutrition Foundation of India, New Delhi to determine whether the inherent genetic potential for growth of children in developing countries is the same as that of American peers. The study was conducted in affluent children of mixed ethnic origin in Delhi Public School, R.K. Puram to study their anthropometric profile. Birth weights of these children were also recorded. It was found that of a total of 4358 children, 29.14% were overweight and 5.32% obese. A strong positive correlation was seen between the birth weights of children and their subsequent growth performance. The percentage of low birth weight deliveries among this group of children was very low (5.6%). It was concluded that the percentage obesity/overweight is on the rise in children and adolescents belonging to the upper strata of the society.

Women’s Nutritional Status

Bone Density in Healthy Women

It is known that women from low income groups are more prone to type 2 osteoporotic fractures. A study was, therefore, undertaken to determine the bone densities (using DEXA) in 138 women (age 35-50 yr) belonging to different socio-economic groups. In addition bone density changes during their perimenopausal phases were also studied. The bone densities at the neck of femur, hip and spine were found to be significantly lower in women from low compared to middle and high income groups. The results suggest that the former have thinner bones (due to their low calcium intake in diet) and poor reproductive health and they require attention.

Fig.4. Seasonal differences in proportions of low birth weight among babies born in Safdarjang Hospital during year 1989 and 1998.
Maternal Body Fat Changes During Pregnancy and its Relation to Neonatal Birth Weight and Fat Mass

Efforts are on to improve birth weights and lower the prevalence of low birth weight. Foetal growth may be influenced by maternal tissue changes like accretion of fat, fat-free mass or body water. A study was undertaken by NIN to assess the relation between the body fat changes in pregnancy (between 20-40 weeks of gestation) and its relation to neonatal fat mass. One hundred and ninety four pregnant women (belonging to low socio-economic strata) at 20 weeks of gestation attending hospital with no obstetric complications were followed up to term. It was observed that the gestational weight gain and fat gain were significantly lower in mothers who delivered low birth weight infants. In addition, low birth weight infants showed higher percentage of fat compared to normal birth weight infants (Fig.5).

**Fig 5. Neonatal fat changes in relation to birth weight of infants**

![Bar chart showing neonatal fat changes in relation to birth weight of infants.](chart)

( ): Number of subjects
Different superscripts indicate significance (p<0.05)

**DIET AND NON-COMMUNICABLE DISEASES**

**Effect of Dietary Alteration on Insulin Resistance**

Insulin resistance is a common metabolic abnormality associated with several diet-related chronic diseases such as obesity, type II diabetes mellitus and hypertension. Dietary fatty acids are known to alter the stored and structural lipids of adipose tissue and skeletal muscle and may affect the insulin sensitivity. Studies were initiated to investigate the effect of dietary alteration of n-6 and n-3 polyunsaturated fatty acids (PUFA) on adipose tissue and skeletal muscle lipid composition, fluidity and insulin action in insulin resistance (sucrose-induced) rat model. The results showed that compared to starch, sucrose feeding significantly increased body weight gain and epididymal fat weight, but alterations of n6/n-3 ratio did not affect body weight gain
and epididymal fat weight. Sucrose feeding resulted in increase in fasting plasma insulin and triglycerides (markers of insulin resistance), which was normalised by reducing the dietary n-6/n-3 ratio.

**Antioxidant and Hypocholesterolaemic Effects of Sesame Oil**

Sesame (*Sesamum indicum* L.) seed is a nutritious food as well as a good source of edible oil. The oil is very stable against deterioration by oxidation. Most of the chemical, biological and physiological properties are due to its unique unsaponifiable constituents viz. Sesamol, Sesamin and Sesamolin. A study was carried out at Central Food Technological Research Institute, Mysore to assess the effect of sesamin on cholesterol metabolism in rats. Findings suggest that sesame oil is more stable than sunflower and groundnut oils not only at room temperature but even when heated at 600°C and 1800°C. Stability of oils blended with sesame oil increased with increase in ratio of the latter. Sesame oil was found to have no adverse effect on growth and development in rats. It had hypocholesterolaemic and hypotriglyceridaemic effect in normal and hypercholesterolaemic rats. The oil inhibited lipid peroxidation, increased the antioxidant enzyme activities and membrane integrity of erythrocytes in cholesterol fed rats.

**Biochemical and Metabolic Studies with Sesame Lignans**

The remarkable stability of sesame oil is attributed to its inherent lignans (sesamol, sesamin and sesamolin) present in its non-glyceride fraction. Studies have shown that lignans inhibit lipid peroxidation *in vitro*. Therefore, studies were carried out to assess the antioxidant properties of sesame lignans *in vivo* by induction of oxidative stress with Fe2+ in weanling W/NIN male rats fed 20% casein-based diet containing different oils. The results suggest that Fe2+ induced oxidative stress was lower in rats fed sesame oil diet compared to those fed groundnut oil diet. However, the antioxidant enzymes showed a marginal increase in rats fed lignan diets. The results confirm the earlier findings from *in vitro* studies that sesame lignans have antioxidant properties.

**Cataract Development**

**Aging and Cataract**

Alpha-crystallin, the major eye lens protein, is constituted by two subunits αA and αB. Both these crystallins have been shown to function as molecular chaperones. They prevent stress-induced aggregation of other proteins and are instrumental in maintaining lens transparency and preventing cataract formation. During aging and cataract, α-crystallin undergoes extensive post-translational modifications such as oxidation, glycation, phosphorylation and truncation in the C-terminus. Study was conducted at NIN to understand the effect of C-terminal truncation of αA-crystallin on its molecular chaperone function. Results have shown that αA-R157stop, a C-terminally truncated α-crystallin has significantly lower chaperone activity than full length α-crystallin against heat as well as UV-B induced aggregation of proteins/inactivation of citrate synthase enzyme activity.
Protective Role of Curcumin in Cataract Development

Cataract is the world’s major cause of blindness and diabetes mellitus is known to advance its onset and progression. Available evidence suggests that oxidative stress and glycation have an important role in cataractogenesis. Recent NIN findings indicate that curcumin, one of the active principles in turmeric, fed at low levels, delayed the onset and maturation (Fig. 6a & b) of galactose-induced cataract in experimental animals. The mechanism of action of curcumin in diabetic cataractogenesis is being deciphered.

Fig. 6a. Cataract progression (Onset)

Fig. 6b. Cataract progression (Maturation)
Role of Aldose Reductase in Cataract Development

Aldose reductase plays an important role in diabetic cataractogenesis. The compounds which inhibit the activity of this enzyme are therefore valuable in delaying/preventing cataract. The extracts of different plants i.e. *amla* (*Emblica officinalis*) and bitter gourd (*Momordica charantia*) fruits and *Tulsi* (*Ocimum sanctum*) leaves were tested for their inhibitory potential against human recombinant and rat lens aldose reductase. Effective inhibitory activity was shown by these extracts.

STUDIES ON MICRONUTRIENTS

Effect of Micronutrients on Intestinal Mucosal Cell Apoptosis

The effect of 50% food and vitamin and 75% protein restriction on mucosal cells of small intestine was studied in rats. The studies showed that protein and vitamin restriction increased apoptosis in villi of small intestine. In vitamin-restricted diet, the crypt height ratio in villi was reduced; similar changes were not observed in other protein or food restricted rats. A ladder pattern was observed in gel electrophoresis in both protein and vitamin restricted groups. Body weight, haemoglobin and serum protein levels were significantly different in vitamin and protein restricted groups.

Selenium in Cereals

An investigation was undertaken this year by NIN to assess the availability of selenium from common foods like rice, wheat, sorghum and red gram dal. The studies carried out in vitro simulating gastric digestion conditions, revealed a good bioavailability of organically bound selenium from the commonly consumed cereals and millets in Indian diet. The results suggest that there may not be selenium insufficiency in Indian populations.

Serum Transferrin Receptor as an Indicator of Iron Status

Validation of ELISA method for estimation of serum transferrin receptors (STR) in adolescent girls and boys was reported last year. Its validation in pregnant women was carried out this year and STR were found to be very sensitive indicators of tissue iron depletion in pregnancy. STR together with haemoglobin can be used for defining iron deficiency in population surveys.

Endemic Fluorosis in Tribals of Mandla

A total of 11791 individuals belonging to 16 blocks of Mandla district in MP were screened. Dental fluorosis was observed in 2% individuals. Maximum sufferers were children and young adults <20 yr. Genuvalgum was observed in 3% and skeletal changes among 1% individuals. The prevalence of skeletal fluorosis was found to increase with age (up to 40 yr) (Fig.7). The prevalence of dental and skeletal fluorosis and genuvalgum was directly proportional to the number of contaminated sources (high fluoride) in the area.
It was also observed that the level of fluoride in the source was directly proportional to the clinical severity or deformity in the area. About 50% urine samples showed high fluoride (more than 2 ppm) content. More than 50% urine samples had high fluoride in the age groups 6-10 and 11-20 yr (Fig.8). The study indicated that fluoride in drinking water is responsible for fluorosis in the study area.

The diet survey indicated that there was significantly low consumption of calcium, iron, vitamin C, carotene, riboflavin, fat and zinc intake in the study area. The intake of cereals was significantly high as compared to RDA. The intake of other food stuffs like pulses, green leafy and other vegetables, milk and milk products, sugar and jaggery were much lower than RDA.
Application of Hazard Analysis Critical Control Point (HACCP) in Mushroom Production

Application of HACCP has become a reference point for international food safety requirements. A study was carried out by NIN to know the extent of HACCP implementation in mushroom industry in developing a safe quality product. The analysis of different batches of mushrooms showed presence of physical, chemical and biological contamination. The Critical Control Points responsible for various contaminants were identified and suitable intervention strategies were suggested to improve the quality of mushrooms. Further, the study indicated that HACCP certification is followed by the industry to satisfy trade regulations rather than to address real hazards associated with mushrooms as a food item.

Metallic Content of Milk and Milkfoods

Sporadic reports on contamination of infant foods with heavy/toxic metals have appeared in newspapers. No standards are yet fixed for levels of heavy metals under the Prevention of Food Adulteration Act (PFA). At the request of DGHS, Government of India, a study was carried out at NIN to analyze 100 market samples of milk and milkfoods (tinned and untinned) for heavy metals like lead, cadmium, tin and arsenic. Levels of cadmium, tin and arsenic were not detectable or were low in all the samples. Lead was measurable in 55 samples and four samples had values more than 1 µg/g indicating an urgent need to fix the standard levels of heavy metals for infant foods under PFA.

Survey of Quality of Market Samples of Edible Oils

There have been reports in press regarding adulteration of edible oils with other edible and/or non-edible oils. A study was conducted by NCLAS to evaluate the quality of market samples of edible oils and fats using physico-chemical parameters. Out of a total of 170 samples (11 types of edible oils) in pouches and sealed containers analyzed, 42 % were found to be adulterated.

Effect of Turmeric on Upper Aerodigestive Tract Cancer

A project was initiated at NIN to identify the high-risk populations for stomach cancer. In vivo nitrosation of administered proline and the effect of inhibitors like turmeric/vitamin C was studied in Wistar rats. The results indicated a dose-related response; the higher dose of turmeric (2 g) exhibited 50% inhibition compared to only 22% at lower 1g dose. The results suggest that it is possible to bring down the level of in vivo nitrosation in humans thus reducing the risk of stomach cancer.
Effect of Turmeric on NMBA Induced Carcinogenesis

Earlier studies on turmeric at NIN have shown that it protects against benzo(a)pyrene and dimethyl benzanthracene induced tumours. In the present study, the effect of turmeric and curcumin on nitrosomethyl benzyl amine (NMBA) (administered for 32 weeks) induced oesophageal and fore-stomach cancer in male WNIN rats was studied. The results of the study indicate that NMBA per se failed to induce significant neoplastic changes within this period. However, it produced proliferative changes when quantitated. Feeding of 1% turmeric after initiation significantly inhibited the proliferative changes. It also exhibited post-initiation inhibitory responses. Feeding curcumin, however, did not produce any inhibitory effects (Fig.9).

Development of an in vivo Model for Genotoxicity

A study was undertaken at NIN this year to investigate the protective role of Alliums (garlic and onion) against cellular damage in rats administered carcinogens, benzopyrene (BP). Cellular damage in liver and kidney tissues was assessed by comet assay. The study demonstrated that prior feeding of Allium reduced DNA damage in tissues due to carcinogen administration (Table 1).

![Fig.9. Effect of turmeric/curcumin on NMBA induced carcinogen abnormalities in male rats](image-url)
Table 1. Assessment of cellular DNA damage due to BP administration and the ameliorative effect of Allium feeding

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Comet Ratio: Diameter/Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0.969 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>BP</td>
<td>0.899 ± 0.65</td>
</tr>
<tr>
<td>3</td>
<td>Garlic 0.1% + Onion 1%</td>
<td>0.986 ± 0.027</td>
</tr>
<tr>
<td>4</td>
<td>Garlic 0.1% + Onion 1% + BP</td>
<td>0.91 ± 0.031</td>
</tr>
<tr>
<td>5</td>
<td>Garlic 0.5% + Onion 5%</td>
<td>0.972 ± 0.034</td>
</tr>
<tr>
<td>6</td>
<td>Garlic 0.5% + Onion 5% + BP</td>
<td>0.92 ± 0.047</td>
</tr>
</tbody>
</table>

Studies on Nutrients and Other Health Promotion Factors

Plant Antioxidants

A detailed investigation was undertaken to estimate the polyphenol and other antioxidant contents in plants from northeast India. Extracts from *Leucos* and *Cessampelos* showed very high levels of tannins, flavonoids and vitamin C and it was found to be related to their antioxidant potential.

Studies on Ginger

Spices like turmeric, cloves and ginger have earlier been shown to have many health benefits. Studies have demonstrated this year that the activity of quinine reductase, a drug metabolising enzyme, is enhanced in rat liver on ginger feeding (Fig.10). This could lead to detoxification of carcinogens. The study is being continued to verify reduction in the formation of urinary mutagens on administration of ginger.

Fig.10. Activity of quinine reductase enzyme in rat liver.
Studies on Physiologically Active Phytonutrients

Foods are not only a source of nutrients but also contain a variety of non nutrient compounds with antioxidant, antimutagenic, anticarcinogenic and anticataractogenic properties.

Tea is a rich source of antioxidants. However, it is not known whether addition of milk to black tea, a practice commonly followed in India, affects its antioxidant activity. NIN studies in vitro and in human volunteers have shown that addition of milk to black tea (a rich source of catechins, the potent antioxidants) may not adversely affect its activity in vitro or the antioxidant status of the subjects consuming it.

OTHER STUDIES

Studies on Obese Mutant Rats

Molecular analysis of WNIN/Ob rats had suggested earlier a possible defect in leptin receptor gene (Ob-R). This year, designing primers (which could amplify around 500 bp in each amplification reaction) sequenced the entire coding region of Ob-R. No apparent change was observed in +/+ and -/- genotypes when compared with published nucleotide sequence. Quantitative RT-PCR on the RNA extracted hypothalamus also did not indicate changes in m-RNA expression between the three genotypes. At the same time, leptin receptor binding data, using brain membranes from these rats showed significantly low binding in the (-/-) genotypes. Detailed analysis of the leptin receptor gene is being undertaken.

Regarding the PCR based DNA fingerprinting using random primers, it was reported earlier that two male specific PCR products specific for WNIN/Ob rats were identified. This year, these products were cloned and the nucleotide sequencing is in progress. Additionally, 5 more random primers could be obtained, two of which could differentiate between the parental WNIN strain and the WNIN/Ob and the other three between WNIN/Ob and the GR-Ob. These are also being cloned and sequenced.

Earlier studies showed oxidative damage in obese mutant rats at an early age with respect to formation of protein carbonyl and lipid peroxides. Anti-oxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase and glutathione S transferase also showed changes in different tissues in one and three month old rats compared to parental strain, WNIN. This year analysis of these enzymes in 9-month old rats showed the same trend, with maximum decrease in liver, except for glutathione peroxidase. The levels of antioxidant enzymes are being estimated in rats of higher age groups and studies on oxidative damage to DNA have also been initiated.

Studies on Wild White Rats

A wild white rat colony with limited production is maintained at NCLAS for the past few years. The uniqueness of this rat with respect to some anatomical features was
described earlier. This year haematological analysis of this rat was carried out for comparison with WNIN rats. Significant differences were observed between wild white rats and WNIN, especially with respect to haemoglobin, RBC and platelet counts. Haemoglobin pattern was also found to be different in wild rats, as revealed by electrophoresis pattern of erythrocyte lysates. While in other laboratory rat strains, haemoglobin β chain showed five bands, only two bands could be seen in wild rats.

Fig.11. Effect of *C.diurnum* leaf powder feeding in egg laying hen on egg production during 24 to 72 weeks

**Vitamin D to Enrich Poultry Feed**

Earlier studies have shown that inclusion of dried *Cestrum diurnum* (CD) leaf powder (a rich source of D₃ glycoside) in the vitamin D₃ deficient poultry feed significantly improved the performance of the broilers. Studies carried out during this year in the egg laying hen indicate that supplementing poultry feed with dried CD leaf powder may improve egg production (Fig.11), increase shelf life and reduce the loss of eggs during transportation. Specific gravity of eggs and thickness and calcium content of egg shells is increased resulting in less breakage.

Values are Mean ± SE of 60 determinations.
Values bearing different superscripts are significantly different between the groups at *p* < 0.05 (ANOVA).

Fig.11. Effect of *C.diurnum* leaf powder feeding in egg laying hen on egg production during 24 to 72 weeks