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HIGHLIGHTS

Being at the forefront of Nutrition Research in the country, the research endeavours of National Institute of Nutrition (NIN) encompassed a wide range of areas in food and nutrition related issues of national importance. The problems that have been dogging the nutritional well-being of population and the emerging epidemiological issues were given considerable importance. While some clinical studies laid emphasis on issues like osteoporosis among women, the lab-based studies looked into a plethora of areas such as, molecular mechanisms of cataract, insulin resistance, ageing process, food compositions, cancer and xenobiotics. On the other hand, efforts were initiated to start a National Dried Blood Spot Facility for vitamin A estimation at NIN, while the community studies looked into different aspects of public health interest like the prevalence of micronutrient deficiencies, iodine deficiency disorders, nutritional status of population in drought-hit states and adolescent obesity.

I. COMMUNITY STUDIES

Several States in India have been experiencing recurring drought conditions during the past few years. The year 2002-03 was no different for many states. On a request from the Ministry of Agriculture, a survey was carried out in nine drought-hit states to assess the impact of drought on diet and nutritional status of the community during May-June 2003. The mean intakes of foodstuffs in almost all the states were low and the households in all these states were not meeting the recommended levels of cereals and millets. Food Security was also not too encouraging among the households. The survey also found that the prevalence of underweight among pre-schools children was more than 50% in all these states, while prevalence of chronic energy deficiency ranged between 26% and 49% among males and 30% and 51% among females.

A large scale, eight-state community based survey was carried out to study the prevalence of micronutrient deficiency disorders involving both clinical and biochemical forms of vitamin A deficiency (Bitot's spots), iodine deficiency disorders and iron deficiency anaemia during this year. The overall prevalence of Bitot's spots was 0.8% and total goiter was 4% (below the WHO cut-off level of 5% to indicate endemicity). About 42% of the households were using non-iodized salt. It was largely lactating and pregnant women, adolescent girls and pre-school children who were found to be affected by anaemia. While IDD registered a decline well below the epidemic level, vitamin A deficiency continued to be a matter of public health concern in many states. The results underscore the need to strengthen the existing national nutrition programmes as well as nutrition education component embedded in them.

An earlier country-wide survey carried out to assess the changes in the prevalence of IDD particularly in the districts with higher levels of endemicity has revealed that the overall prevalence of total goiter registered a significant decline from 14-69% during 1984-94 to 3-40% this year, especially in the North-Eastern region of the country.

The fact that prevalence of obesity among adolescents is increasing, attracted attention and prompted a study to assess the prevalence of over-weight and obesity in urban adolescent school children. The study proved that the prevalence was higher among urban school children than their rural counterparts.

Yet another study established that consumption of breakfast was an important factor affecting cognitive functions especially attention concentration and immediate recall memory of the students. At the same time, it was also observed that the academic performance of regular breakfast eaters was better than the non-eaters.

II. CLINICAL AND PHYSIOLOGICAL STUDIES

Osteopororsis results in increased bone fragility and may lead to fracture of spine, hip and arm which is both an active as well as an early symptom. Research findings have revealed that Indian women experience early onset of osteoporosis as compared to their Western counterparts. Hence, they are more vulnerable to the problem.

Most of the Indian women belonging to the underprivileged sections of the society subsist on a diet low in calories, proteins as well as calcium. These women are seen to breastfeed their infants for prolonged period of time (>1 year). It is possible that their dietary calcium is inadequate for bone accretion during the recovery period and hence there may be a mobilization of calcium from mother's skeleton leading to transient reduction in bone mineral density (BMD). Studies are being carried out to establish peak BMD reference values for both men and women and also to assess the prevalence of osteopenia and osteoporosis in Indian population groups in a multicentric ICMR Task Force Study. Another study is being conducted to assess the extent of loss of bone mass during post-partum period and also to find out if low calcium intakes are adequate for restoration of bone density. Conservation of calcium seemed to occur either through increased absorption or reduced excretion, or both. These compensatory mechanisms were observed to offset the breast milk calcium loss only in those women who enjoyed better nutritional status (in terms of body weights and BMI). The link between body weight and BMI with peak bone mass will be explored in further studies.

III. BASIC STUDIES

Assessment of vitamin A status in the population groups is a prerequisite to successful prevention and control of vitamin A deficiency disorders. A technology using the process of collecting dried blood spot (DBS) on filter paper and later analyzing for vitamin A using HPLC has been developed and made available at NIN. This National Facility was initiated with the support from Micronutrient Initiative and MOST, New Delhi.

The enzyme, aldose reductase (AR) in lens has been a drug target because of its involvement in the development of secondary complications of diabetes including cataract. A study was carried out to assess the inhibition of AR by the constituents of Emblica officinalis *in vitro* and in lens organ culture. It was found that aqueous extract of E.officinalis inhibited rat lens AR and recombinant human AR. The hydrolysable tannoids of E. officinalis were found to be responsible for AR inhibition. In an alternative approach, antiglycating agents (MAB1) have been worked out for delaying the onset of opacification of lens.

A study was carried out to assess the effect of calorie restriction with/without micronutrient deficiency on oxidative stress and ageing. The findings of the study established the beneficial effect of calorie restriction *per se* in protecting animals against oxidative stress and hyperinsulinemia.

Resistin, a cysteine rich adipocytokine, has shown to be implicated as a link between obesity and type 2 diabetes in mouse. Role of resistin in human was debatable. A study was carried out to characterize the structure of human resistin which revealed a reversible shift in secondary structure as a function of concentration and time. The level of expression of resistin gene in human will modulate the higher order structure and by implication its function.

Studies were carried out to investigate the effects of increasing dietary long chain n-3 PUFA from fish oil on membrane lipid composition and insulin sensitivity in skeletal muscle and adipose tissue of sucrose induced insulin-resistant rats. The results showed that replacement of 0.5en% long chain n-3 PUFA (n-6/n-3 ratio = 10) prevented sucrose induced insulin resistance by increasing peripheral insulin sensitivity.

IV. FOOD COMPOSITION AND NUTRIENT AVAILABILITY

Rice, being the staple food in many parts of India, research guidelines for improvements in yield is an important process. As a result, new varieties are produced by the scientists and subsequently they enter the markets. The protein quality depends on their essential amino acid composition, hence a study was conducted on varieties of rice by analysing them for protein and amino acids content before and after polishing and were compared to the values documented in NIN's publication Nutritive Value of Indian Foods (NVIF). Results indicated that 8-10% polishing of rice decreased protein content by about 5%. In most cases, it was observed that the protein values of new rice varieties were higher than the earlier values reported in NVIF, with out any change in lysine content.

V. CANCER AND XENOBIOTICS

Antimutagenic and antigenotoxic potential of ginger was clearly established in a study. Another study was conducted on the patients suffering from upper gastrointestinal tract cancers. Estimation of *in vivo* nitrosation potential after administering proline was carried out. The metabolites of nitroproline were found to be significantly higher.

In the field of social drug epidemiology, an educational intervention strategy comprising both print and traditional folk-form in Andhra Pradesh (Harikatha) was used to sensitize general public on the issues of Rational Usage of Drugs. Also, a rapid screening procedure (*in vitro* and *in vivo*) was developed to assess the antioxidant activity of some herbal medicines.

VI. FOOD SAFETY

The institute is frequently approached by various agencies including governmental organizations to carry out community-based studies on public health issues. A rapid survey carried out in the villages of Bhandara district of Madhya Pradesh has revealed that several people were affected with toxicity-related illness on consuming Khesari dal (Lathyrus sativus). Another study in the tribal districts of Orissa showed that food samples were contaminated with aflatoxins and some other heavy metals including lead, cadmium, arsenic and mercury. In yet another study carried out in some villages of Nawadha district of Bihar, it was found that high fluoride concentration in drinking water was leading to vitamin D deficiency causing bone deformities in young children. Appropriate strategies to combat the problem were suggested.

VII. PATHOLOGY

A study was carried out to determine the effect of vitamin A restriction and supplementation on drug induced apoptosis of rat intestinal mucosal cells. It was observed that riboflavin and folic acid supplementation helps in preventing DNA damage, mutations and the occurrence of cancer as well as chemotherapy restricted adverse effects.

VIII. NCLAS

DNA finger printing of the obese mutant rats using random primers yielded a fairly constant DNA fingerprint for the GR-Ob strain. Similar pattern was not found in WNIN/Ob strain and hence alternative techniques to obtain results are being explored. Also, genetic typing of obese mutant rats using microsatellite markers was carried out. Out of 100 markers proposed to be screened, 60 primers spanning a majority of the chromosomes have been completed.

IX. PRE-CLINICAL TOXICOLOGY

The existing Pre-clinical Toxicology expertise in the Institute gained new emphasis and continued to carry out various service activities assigned to it. A research study was also taken up to carry out toxicity and allergenicity evaluation of recombinant Hepatitis B vaccine in mice and guinea pigs.

Thus research studies carried out in a wide array of fields epitomise the Institute's consistent zest in exploring newer frontiers in the filed of nutrition, while addressing the current issues in a holistic approach. These research achievements would not have been possible without the co-operation extended by all the staff of NIN, with whose unstinted support the institute forges ahead to face newer challenges.

I. COMMUNITY STUDIES

1. DIET AND NUTRITIONAL STATUS IN DROUGHT AFFECTED AREAS IN THE COUNTRY

Investigators : K.Vijayaraghavan, G.N.V.Brahmam, K.Venkaiah, M.Vishnuvardhana Rao, N.Arlappa, K.Mallikharjuna Rao, Ch. Gal Reddy, Sharad Kumar, M. Ravindranath and Anjali Singh

Duration : Six months

Date of completion : October 2003

Several States in India have been experiencing recurrent drought conditions during the past few years due to inadequate or failure of rainfall or delay in the onset of monsoon. Natural calamities like drought/famine or floods are known to affect adversely the country's economy in terms of drop in agricultural production and agro-based industrial output leading to rural unemployment, decrease in purchasing power, migration to urban areas, reduced household food and nutrient security, resulting in increase in the prevalence of undernutrition and morbidities in the community. The year 2002-03 has been another year of drought and the Government of India declared nine States viz., Andhra Pradesh, Karnataka, Tamil Nadu, Madhya Pradesh, Maharashtra, Rajasthan, Gujarat, Chattisgarh and Orissa as drought affected. A rapid survey, was therefore, carried out at the request of the Ministry of Agriculture, Government of India, during May-June 2003, to assess the impact of drought on diet and nutritional status of the community in the drought affected areas.

The specific objectives of the study were,

- To assess the nutritional status in terms of food and nutrient intake, anthropometry, and prevalence of clinical forms of undernutrition, of the communities living in the severely drought affected areas of the above States,
- To assess the household food security status, coping mechanisms adopted and extent of participation in the drought relief activities and other developmental programmes during current drought,
- To assess the impact of drought, if any, on the vulnerable segments of the population such as landless labourers, marginal farmers and Scheduled Caste and Scheduled Tribe communities and
- To suggest suitable remedial measures for the control of ill effects of drought on nutritional status of the population.

SAMPLING

In each State, the survey was carried out in two severely drought affected (based on rainfall) districts. In each district, two severely affected blocks were selected. From each of the selected blocks, 5 villages were selected randomly. In each village, 60 households (HHS) were covered by adopting cluster-sampling procedure. For this purpose the village

was divided into five geographical areas, based on natural groups of households, one of which consisted of schedule castes/ scheduled tribes. From each of these areas, a cluster of 12 households were covered with a random start. Thus, in each State, information on household demographic and socio-economic particulars as well as nutritional status of all the available individuals was collected from 1200 households. Data on household food security and coping strategies adopted during drought was collected in a sub-sample of 400 HHs. In a sub-sample of 100 HHs, from these individual food and nutrient intakes were assessed by 24-hour recall method of diet survey.

In the State of Rajasthan, at the request of Ministry of Agriculture, survey was carried out in a total of 3 districts.

The salient findings of the survey in each of the States covered are as follows:

ANDHRA PRADESH

- In the areas surveyed there was about 80-90% of deficit in the rainfall.
- A reduction of 25-30% in the area sown and 75-80% in the yield was reported in both the districts.
- About 20% of the villages surveyed reported scarcity of drinking water.

Participation of Households in Poverty Alleviation Programmes

About 41% of the households were participating in *Sampoorna Gramina Rojgar Yojana* (SGRY). Targeted Public Distribution System (TPDS) was availed by 65% of the HHs. About 16% of the HHs were availing pension (old age/widow/disabled) scheme and 6.9% were participating in PDS. About 2-3% of HHs were availing *Annapurna* and *Anthyodaya Anna Yojana* (AAY). Majority of them were participating in supplementary feeding programmes such as Integrated Child Development Services (ICDS: 75%) and Mid day Meal (MDM: 91%).

Food and nutrient intake

- Average daily household consumption of various foods was below recommended dietary allowances (RDA) levels. However, the intakes of all the foodstuffs except other vegetables were higher than the intakes reported in the previous drought survey (year 2000).
- Intake of all the nutrients was reported to be lower than the RDA. The extent of deficit ranged from a minimum of 24% in case of protein to a maximum of 69% of RDA for iron. However, the proportion of households consuming various nutrients in levels less than 50% of RDA decreased marginally as compared to previous drought survey.
- None of the households surveyed were found to be consuming energy below 500 kcal/ CU/day, a level suggestive of starvation diet.

Nutritional status

- Prevalence of underweight (weight for age < Median-2 SD) was 69% and that of severe underweight (<-3SD) was 23% among preschool children as against 63% and 18% respectively, observed in previous drought.
- About 55% of the preschool children were stunted, compared to 40% reported earlier.
- Prevalence of overall wasting during the current survey was about 24%, which was lower than the figure reported (31.8%) in the previous drought.

- The overall prevalence of undernutrition in adults in terms of Chronic Energy deficiency (CED) (BMI <18.5) was significantly higher among both males (50.2% vs 41.3%) and females (55.9% vs 50.1%) during the current drought, compared to previous drought.

Impact of drought on households

- Only about 32% of households reported insufficiency (of 25%) in cereal and millet consumption, while more than 80% of the households reported insufficiency in the consumption of income elastic and protective foods like pulses, vegetables, milk and milk products and fats & oils. Relatively higher proportion of households reporting minimal or no change in the consumption of staple foods during current drought could be attributed to the supply of food grains through TPDS/PDS at subsidized rates.
- Common coping strategies reportedly adopted by the households during the current drought were consumption of low cost foods (71%), borrowing cash/food from neighbours (69%), reduction of food consumption (66%), disposing household assets (29%) and migration (20%).

Nutritional status vs socio-economic variables

- Prevalence of underweight (<Median - 2SD) was marginally higher among preschool children belonging to SC/ST Communities and those belonging to HHs engaged in agricultural labour.
- Significantly higher proportion of children from SC/ST households were stunted (61%) compared to those belonging to other communities (50%).

The prevalence of CED was significantly ($p<0.01$) higher among the adults belonging to SC/ ST communities, marginal farmers, agricultural labourers and non-agricultural labourers.

CHATTISGARH

- More than 80% of the villages reported a deficit of 75-80% in rainfall as compared to normal period.
- A reduction of 80% in the area sown and about 80-90% reduction in the yield of various crops were reported in both the districts.
- While none of the villages surveyed in Durg district had scarcity of drinking water, about 75% of the villages surveyed in the district of Bastar reportedly had drinking water scarcity.

Participation of Households in Poverty Alleviation Programmes

- About 67% of the households were participating in *Sampoorna Gramina Rojgar Yojana* (SGRY) scheme.
- About 38% of the households were reportedly availing TPDS, 8% of the households were availing pension and 12% were participating in PDS and 8% were availing AAY.
- Majority of the eligible households, in general, were participating in supplementary feeding programmes such as ICDS (60%) and MDM (72%) respectively.

Food and nutrient intake

- Average daily household consumption of various foods was below the recommended level.
- Intakes of all the foodstuffs except green leafy vegetables were lower than the NNMB surveys 2000-01 for the State of Madhya Pradesh.
- Average daily intake of all the nutrients (per CU/day) was reported to be lower than the RDA. The extent of deficit ranged from a minimum of 41% in case of niacin to a maximum of 78% of RDA for free folic acid.
- The proportion of households consuming various nutrients in levels less than 50% of RDA was maximum for free folic acid, riboflavin and iron (about 90% each).
- None of the households were found to be consuming energy below 500 kcal/ CU/day, a level suggestive of starvation diet.

Nutritional status

- Prevalence of underweight (weight for age <Median -2SD) among preschool children was 68% and that of severe underweight was 20%. It was significantly lower (67.9%) ($p<0.05$) as compared to the levels reported for the State of Madhya Pradesh (74.2%) by NNMB surveys (2000-01).
- Prevalence of overall wasting during current survey (15.6%) was lower than the figure reported (16.4%) by NNMB surveys (2000-01) for the State of Madhya Pradesh.
- About 62% of preschool children were stunted, while the prevalence of severe stunting was about 28% which was significantly lower ($p<0.05$) as compared to NNMB surveys.
- Prevalence of CED (BMI <18.5) among adult males (40.3%) and females (43.8%) during the current drought was comparable to that reported by NNMB survey 2000-01 (42.9% male; 42% female for the State of Madhya Pradesh).

Impact of drought on households

- About 84% of the HHs reported insufficiency in the consumption of vegetables to the extent of 25-50%, followed by 72% for fats & oils, 60% for roots & tubers, 48% for pulses and 45% for milk & milk products. Relatively small proportion of HHs reported insufficiency in case of cereals and millets perhaps due to the supply of food grains by TPDS/PDS at subsidized rates.
- Common coping strategies reported by the HHs during the current drought were consumption of low cost foods (96.3%), reduction in consumption of foods (95.5%), use of food stocks (95.2%) and availing Government assistance (71.7%).

Nutritional status vs socio-economic variables

- The prevalence of underweight (< Median -2SD) among preschool children was comparable between households belonging to different occupational groups, SC/ST communities and those with varying extent of land holdings.
- The proportion of children with stunting was significantly higher ($p<0.05$) among households engaged in non-agricultural labour (72%) as compared to those belonging

to other occupations (59%). The prevalence of overall stunting was significantly higher ($p<0.05$) among marginal farmers (64%) as compared to small farmers (49.1%).

¨ The overall prevalence of CED among adults was significantly higher ($p<0.05$) in SC/ST Communities (45%) as compared to others (39%), in non-agricultural labour (46.9%) as compared to other occupations (38%) and among marginal farmers (48%) as compared to small farmers (40%).

GUJARAT

© The areas surveyed were chronically drought affected for the past three years. During the current year 30% of villages reported no rainfall at all, while about 70% of the villages reported 75-100% reduction as compared to rainfall in normal period.

© Only about 50% initiated agricultural operations in wet lands while only 20% did so in dry lands. There was about 85-100% overall reduction in the crop yield.

© Severe scarcity for drinking water was reported in majority of the villages surveyed. However, in about 50% of the villages, the Government made arrangements for supply of drinking water through tankers.

© There was a severe scarcity for fodder in all the villages surveyed. In only 5 villages (25%) the Government had established cattle camps.

Participation of Households in Poverty Alleviation Programmes

¨ About 69% of the households were reportedly participating in SGRY scheme.

¨ Only 25% of the households availed TPDS, while 16% were participating in AAY scheme.

¨ The proportion of households availing either old age / widow/ disabled pension or *Annapurna* scheme was found to be negligible (1%).

¨ About 76% of the eligible households, were participating in supplementary feeding programmes such as ICDS and MDM.

Food and nutrient intake

– The average daily household consumption of various foods except roots & tubers and milk & milk products was below the recommended levels.

– The levels of consumption of cereals & millets, the major staple during the current drought was less than that observed during the previous drought survey (2000).

– The intakes of nutrients such as energy, riboflavin, vitamin A, free folic acid and vitamin C were lower than the RDA.

– The extent of deficit in the intakes ranged from a low 2% in case of total fat to a maximum of 65% of RDA for vitamin A.

– The intakes of energy, protein, total fat and vitamin C were less than the figures reported in 2000 drought survey, indicating a decrease in the intakes of the major nutrients during the current drought.

– None of the households were found to be consuming energy below 500 kcal/ CU/day, a level suggestive of starvation diet.

Nutritional status

- Overall prevalence of underweight (weight for age <Median -2 SD) among preschool children was 65% and that of severe underweight was 20%.
- About 62% of preschool children were stunted as compared to 49% during previous drought. Prevalence of severe stunting was significantly ($p<0.05$) higher (34.3%) than the figures reported (21.9%) during the year 2000.
- Prevalence of overall wasting during the current survey was about 12%, which was lower than the figure reported by the previous drought (29.5%).
- The overall prevalence of CED (BMI < 18.5) was significantly lower ($p<0.01$) among both males (34.2%) and females (29.5%) during the current drought as compared to the figures reported during the previous drought (48.7% males; 35.8% females).

Impact of drought on households

- About one-fourth of the households (23%) reported insufficiency in cereal and millet consumption to the extent of 25%. Similarly, about 50-60% of the households reported 25-50% insufficiency in income elastic foods like pulses (57.2%), fats & oils (52%) and milk & milk products (50.5%). Relatively lower proportion of households reported insufficiency in the consumption of staple food (23%) during current drought perhaps due to the supply of food grains by TPDS/ SGRY at subsidized rates.
- Coping strategies reportedly adopted by the households during the current drought were borrowing cash/ food grain (95%), use of food stocks/ savings (79%), obtaining government assistance (57.6%) and reduction in food consumption (26%).
- About 69% of the households were participating in 'food for work' programme.

Nutritional status vs socio-economic variables

- No significant difference was observed between the prevalence of under weight, stunting and wasting between communities.
- Prevalence of CED was observed to be higher among males (48%) and females (39%) belonging to SC/ST communities as compared to others (33% and 28% respectively). However, the differences were statistically significant in females only.
- Prevalence of CED was higher among adults belonging to landless and agricultural labourers as compared to others.

KARNATAKA

- The average rainfall was less than normal (by about 75-90%) in both the districts for the last four years.
- A reduction of about 75% - 90% in the yield was reported in both the districts
- In about 90% of the villages surveyed, scarcity of drinking water was reported. Community water supply scheme was in operation in 50% of the villages surveyed in Gulbarga and 90% of the villages in Kolar district.

Participation of Households in Poverty Alleviation Programmes

- About 10% of the households were participating in *Sampoorna Gramina Rojgar Yojana* (SGRY) scheme.
- About 71% of the HHs interviewed were participating in TPDS, followed by 7.3% in Pension Scheme (Old age/ Widow), 4% in PDS and 2.2% in AAY.
- Eighty three percent of the eligible households were participating in supplementary feeding programmes such as ICDS and Mid Day Meal Programme (MDM).

Food and nutrient intake

- Average daily household consumption of various foodstuffs, except for cereals & millets and pulses was below the recommended levels as well as the figures reported by NNMB during non-drought period (2000-01).
- The consumption of protective foods such as green leafy vegetables, other vegetables, fruits, milk & milk products decreased markedly during the current drought as compared to non-drought periods.
- In general, the diets were deficient in protein, energy, iron, vitamin A, riboflavin, niacin, vitamin C and free folic acid.
- The proportion of HHs consuming various nutrients in levels <50% of the RDA was maximum for vitamin A (93%), followed by vitamin C (80%), free folic acid (61%), riboflavin (55%) and iron (51%).
- None of the HHs were found to be consuming energy below 500 kcal/CU/day, a level suggestive of starvation diet.

Nutritional status

- Prevalence of underweight (weight for age <Median -2 SD) among preschool children was about 58%, while that of severe underweight (weight for age <Median –3SD) was about 20%, and were comparable with that reported by NNMB.
- Prevalence of CED (BMI <18.5) during the current drought was higher ($p<0.01$) among both males (48.6% vs 36.2%) and females (51.3% vs. 39.7%) during current drought, as compared to that reported by NNMB.

Impact of drought on households

- About 48% of the households reported insufficiency in the consumption of cereals and millets.
- Food insecurity was reported to be more with respect to income elastic foods like milk & milk products (61.2%), fats (78.1%) and pulses (78.4%).
- About half of the HHs reportedly had about 50% insufficiency for vegetables and roots & tubers.
- Consumption of milk and milk products was nil in 46% of the households.
- The relatively higher proportion of HHs reporting minimal or no change in consumption of staple food could be attributed to the supply of food grains through TPDS/PDS at subsidized rates.

- Coping strategies adopted by the households during the current drought were, reduction in food consumption (96.7%), obtaining government assistance such as TPDS/ AAY (80.5%), consumption of low cost foods (64.8%), utilization of available food stock/ savings (24.9%), borrowing of cash/food grains from neighbours (22.4%), migration (21.5%) and disposal of HH assets (5.3%).
- Out of 402 HHs surveyed, only 10% of HHs had participated in Food for Work Programme.

Nutritional status vs socio-economic variables

- No significant association was observed between the overall prevalence of underweight in preschool children vs. community, land ownership and major occupation of the head of the household. However marginal differences were observed between SC, ST 61% and other communities 56%.
- The overall prevalence of CED among males was significantly higher ($p<0.05$) in SC/ST Communities (about 54%) as compared to those belonging to BC and other communities (45%).
- No significant differences in the prevalence of CED were observed among households with varying extent of land holdings or major occupations.

MADHYA PRADESH

- About 70-75% of deficit in the rainfall was reported in the area surveyed.
- Seventy percent of the villages did not cultivate any crops in the wet land, while in about 60-70% villages dry land cultivation was taken up during the year. There was a reduction of 75-80% in the yield of various crops.
- Scarcity of drinking water was reported in all the villages surveyed.

Participation of Households in Poverty Alleviation Programmes

- Fifty one percent of the households were participating in SGRY scheme.
- Forty seven percent of the households were availing TPDS, 8.3% were participating in PDS, 4% of the households were availing pension schemes and 8.3% were availing AAY.
- Majority of the eligible households were participating in supplementary feeding programmes such as ICDS (59%) and MDM (79%) respectively.

Food and nutrient intake

- Average daily household consumption of various foods was below recommended levels as well as the figures reported by NNMB for the State during 2000-01.
- Consumption of all the nutrients was reported to be lower than the RDA as well as the figure reported by NNMB. The extent of deficit ranged from a minimum of 22% in case of thiamin to a maximum of 71% of RDA for vitamin A.
- Proportion of households consuming various nutrients in levels less than 50% of RDA was maximum for vitamin A, free folic acid and riboflavin (about 88% each), followed by iron (78%), calcium (74%) and vitamin C (72%).

- None of the households surveyed were found to be consuming energy below 500 kcal / CU/day, a level suggestive of starvation diet.

Nutritional status

- The prevalence of underweight (weight for age <Median -2SD) among preschool children was 69% and that of severe underweight (<-3SD) was 25%.
- Seventy one percent preschool children were stunted, a level comparable to that reported by NNMB for the State (72%).
- Prevalence of wasting (indicator of current undernutrition) was 15% which was comparable to that reported by NNMB (16.4%) (2000-01).
- Prevalence of CED (BMI <18.5) was significantly lower ($p<0.01$) among both adult males (34.8%) and females (33.8%) during the current drought as compared to those reported by NNMB (42.9% males and 42% for females) for the State (2000-01).

Impact of drought on households

- Only 10% of households reported sufficiency with respect to all major foods. A majority of the households (61-75%) reported (about 50%) insufficiency in the consumption of various foods such as pulses, vegetables and roots & tubers. Insufficiency (>75%) was reported with respect to milk & milk products and fats & oils by about 70% to 80% of the HHs.
- Coping strategies reportedly adopted by the households during the current drought were reduction in the food consumption (88.5%) and obtaining government assistance (60%).

Nutritional status vs socio-economic variables

- Prevalence of underweight (<Median -2SD) was significantly more ($p<0.05$) among children belonging to agricultural labourer and SC/ST communities as compared to others.
- No significant differences were noticed in the prevalence of overall stunting, between communities, extent of land holdings and major household occupations.
- Prevalence of CED among adult males was significantly higher ($p<0.05$) among SC/ST communities (37%) as compared to others (27%) but was similar among HHs with different extent of land holdings, or major occupations.

MAHARASHTRA

- About 50-75% of deficit in the rainfall was reported in the area surveyed.
- There was a reduction of 75% to 90% in the yield of various crops in both the districts.
- Half of the villages surveyed reportedly had scarcity of drinking water.

Participation of Households in Poverty Alleviation Programmes

- Twenty eight percent of the households were participating in *Sampoorna Gramina Rojgar Yojana* (SGRY) scheme.

- TPDS was availed by 47% of the households followed by PDS 6.8%. AAY 5% and Pension 1.6%.
- More than half of the eligible households in general were participating in supplementary feeding programmes such as ICDS (60%) and MDM (55%) respectively.

Food and nutrient intake

- Average daily consumption of various foods by the households was below the recommended levels, and was comparable with that reported by NNMB survey (2000-01) for the State.
- Intake of all the nutrients except thiamin was lower than the RDA. The extent of deficit ranged from a minimum of 18% in case of niacin to a maximum of 56% of RDA for free folic acid.
- Proportion of HHs consuming less than 50% of RDA was maximum (65.5%) for vitamin A, followed by vitamin C (63.5%), free folic acid (62.5%), calcium (60%), riboflavin (48%) and iron (41%).
- None of the households were found to be consuming dietary energy below 500 kcal/ CU/day, a level suggested to starvation diet.

Nutritional status

- Prevalence of underweight (weight for age <Median-2SD) among preschool children was 63% and that of severe underweight was 16.8%.
- About 45% of preschool children were stunted compared to 38.1% reported by NNMB.
- The overall prevalence of wasting during the current survey was about 23%, which was lower than the figure reported by NNMB survey 2000-01 for the preschool children in the State.
- Overall prevalence of CED (BMI <18.5) among adults was comparable in males (46%) and females (47%), which was marginally higher than that reported during NNMB survey 2000-01 (41% and 45% respectively).

Impact of drought on households

- Sixty four percent of households reported insufficiency in cereal and millet consumption. Food insecurity was reported to be more with respect to income elastic and protective foods like milk & milk products (52.5%), fats (67.5%), vegetables (67.3%) and roots & tubers (67%).
- Coping strategies reportedly adopted by the households during the current drought were, reduction in food consumption (97.2%), consumption of low cost foods (79%), borrowing cash/food from neighbours (16%), disposing of household assets (9.5%) etc.

Nutritional status vs socio-economic variables

- Significantly higher proportion ($p<0.05$) of children from SC/ST HHs and those from landless HHs had underweight and wasting compared to others.
- Prevalence of CED was significantly higher ($p<0.05$) among SC/ST communities (males: 50% and females: 57%) as compared to those belonging to BC and other communities (males: 40% and females: 36%).

- No significant differences in the prevalence of CED were observed among HHs with different extent of land holdings.

ORISSA

- There was about 70-90% of deficit in the rainfall in the areas surveyed
- Extent of reduction in the yield of various crops ranged from 35-100%.
- None of the villages surveyed reportedly had scarcity of drinking water, as adequate number of bore wells were provided by the Government.

Participation of Households in Poverty Alleviation Programmes

- Twenty three percent of the households were participating in *Sampoorna Gramina Rojgar Yojana* (SGRY) scheme.
- TPDS was availed by 27% of the households, 13% of the households were availing pension and 1% were availing *Annapurna* and 6% were availing AAY.
- A majority of the eligible HHs were participating in Mid Day Meal (MDM) (77%), while it was only to the extent of 37% in ICDS.

Food and nutrient intake

- Average daily household consumption of various foods was below the recommended levels except for roots & tubers.
- Intakes of protective foods such as green leafy vegetables and other vegetables were lower than that reported by NNMB for the State of Orissa (2000-01).
- Intake of all the nutrients except niacin was observed to be lower than the RDA. The extent of deficit as compared to RDA ranged from a minimum of 27% in case of vitamin C to a maximum of 80% of RDA for total fat.
- Proportion of households consuming less than 50% of RDA for various nutrients was maximum for iron (94%) followed by riboflavin (92%), fat (78.5%), free folic acid (66%), calcium (62%), vitamin A (57%) and vitamin C (50%).
- None of the households were found to be consuming dietary energy below 500 kcal / CU/day, a level suggestive to starvation diet.

Nutritional status

- Prevalence of underweight (weight for age < Median -2 SD) among preschool children was 66% and that of severe underweight (<Median –3SD) was 21% among preschool children, was similar to that reported by NNMB for Orissa.
- Fifty five percent of preschool children were stunted, compared to 59% reported by NNMB for the State (2000-01).
- Prevalence of overall wasting during the current survey was about 21% which was comparable to figure reported by the NNMB for the State of Orissa (19%).
- The overall prevalence of CED (BMI < 18.5) was significantly higher among both males (43.7% vs 36.6%) and females (49.6% vs 46%) during the current drought as compared to NNMB survey (2000-01).

Impact of drought on households

- Forty six percent of the households reported insufficiency to the extent of 25% in the consumption of cereals and millet. Another 40% of the households reported 50%

insufficiency in pulses and 34% of the HHs had 75% insufficiency in consumption of fats & oils. Relatively higher proportion of households reporting minimal or no change in the consumption of staple foods during current drought could be attributed to the supply of food grains by TPDS/PDS at subsidised rates.

- Coping strategies reportedly adopted by the households during the current drought were consumption of low cost foods (80%), reduction of food consumption (73%), borrowing cash/food from neighbours (54%), obtaining government assistance (44%), use of food stocks (27%) and migration (24%).
- Thirty one percent of the eligible households reportedly benefited from TPDS, while about 21% of the eligible households were benefited from food for work programme.

Nutritional status vs socio-economic variables

- Prevalence of severe underweight (<Median - 3SD) was significantly higher ($p<0.05$) among children belonging to SC/ST communities, while overall nutrition (<Median – 2SD) was significantly higher ($p<0.05$) among landless agricultural labourers.
- Significantly higher proportion of children ($p<0.05$) from SC/ST households were severely stunted (28%) compared to those belonging to other communities (21%).
- Prevalence of CED among adults was significantly higher ($p<0.05$) in SC/ST Communities (54%) as compared to others (38%).
- The prevalence of CED was significantly higher ($p<0.05$) among those belonging to non-agricultural labour as compared to other occupational groups.

RAJASTHAN

- There was 80-90% deficit in the rainfall in the areas surveyed
- Complete failure of crops was reported in a majority of the villages surveyed.
- One third of the villages surveyed reported scarcity of drinking water.

Participation of Households in Poverty Alleviation Programmes

- Fifty seven percent of the households were participating in *Sampoorna Gramina Rojgar Yojana* (SGRY).
- Targeted Public Distribution System was availed by 30% of the households, 57% were participating in PDS, 3% of the households were availing Pension, 4% were availing *Anthyodaya Anna Yojana* (AAY) and 2% were availing *Annapoorna* Scheme.
- More than half of the eligible households were participating in supplementary feeding programmes such as Integrated Child Development Services (ICDS) and Mid Day Meal (MDM).

Food and nutrient intake

- Household consumption of various foods barring roots & tubers was below the recommended levels.
- Consumption of pulses & milk was considerably less compared to previous drought (2000).
- Intake of all the nutrients except protein, calcium, thiamin and niacin was lower than the recommended dietary allowances (RDA). The extent of deficit ranged from a minimum of 16% in case of iron to a maximum of 79% of RDA for vitamin A.

- The proportion of households consuming various nutrients in levels less than 50% of RDA was maximum for vitamin A (96%) and vitamin C (63%) as compared to other nutrients.
- None of the households surveyed were found to be consuming energy below 500 kcal/ CU/day, a level suggestive of starvation diet.

Nutritional status

- Prevalence of underweight (weight for age < Median -2 SD) was 66% and that of severe underweight was 23% among preschool children.
- About 31% of preschool children were severely stunted which was marginally higher than the figure of 27% reported during earlier drought.
- The prevalence of wasting during the current survey was 15.4%, which is lower than the figure reported (26%) during the previous drought.
- Prevalence of chronic energy deficiency (CED) (BMI <18.5) was less (40%) during the current drought as compared to previous drought (43.3%).

Impact of drought on households

- Thirty three percent of households reported insufficiency to the extent of 25% in the consumption of cereal and millet, pulses and milk & milk products. Relatively higher proportion of households reportedly had no change in the consumption of staple food during current drought, which could be due to the supply of food grains through Food for Work TPDS/PDS at subsidized rates.
- Coping strategies reportedly adopted by the households during the current drought were borrowing cash/food from neighbours (48%), obtaining government assistance (28.3%) and reduction in consumption of food (24.5%).

Nutritional status vs socio-economic variables

- The prevalence of underweight, stunting and wasting was significantly ($p<0.05$) higher among preschool children belonging to SC/ST communities, than those of other communities.
- Similarly, the overall prevalence of CED among adults was significantly higher ($p<0.05$) among those from SC/ST communities as compared to others (48% vs 35%), and among those belonging to landless households (44%) as compared to marginal farmers (40%) and small farmers (38%).

TAMIL NADU

- More than 75% of the villages had 80-90% deficit in rainfall.
- About 70% reduction in the area sown and 80-90% reduction in the yield was reported in both the districts.
- Fifty percent of the villages surveyed reported scarcity of drinking water.

Participation of Households in Poverty Alleviation Programmes

- Nine percent of the households were participating in *Sampoorna Gramina Rojgar Yojana* (SGRY) scheme.
- TPDS was availed by 66% of the households. About 6.0% of the households were availing pension (old age / widow / disabled), 7% were participating in PDS and 4.7% were availing AAY.

- Majority of the eligible households were participating in supplementary feeding programmes such as ICDS (69%) and MDM (88%) respectively.

Food and nutrient intake

- Consumption of various foods except pulses & legumes were less than RDA. The consumption of cereals & millets and pulses & legumes were higher during current drought as compared to the figure reported by NNMB (2000-01).
- Consumption of all the nutrients except thiamin and niacin were reported to be lower than the RDA. The extent of deficit ranged from a minimum of 32% in case of protein to a maximum of 83% of RDA for vitamin A.
- Proportion of households consuming less than 50% of RDA for various nutrients ranged from a maximum of 97% for vitamin A to a low 3% for thiamin and niacin.
- None of the households were found to be consuming energy below 500 kcal/ CU/day, a level suggestive of starvation diet.

Nutritional status

- Prevalence of underweight (weight for age <Median -2SD) among preschool children was 53% and that of severe underweight was 11% as compared to 52.6% & 15.1% reported by NNMB.
- Forty percent of preschool children were stunted which was comparable to that of NNMB survey.
- Prevalence of overall wasting during the current survey was about 17% which was lower than the figure reported by NNMB survey (2000-01) (19%).
- The overall prevalence of CED (BMI < 18.5) was significantly lower ($p<0.01$) among adult males (25.8%) during the current drought as compared to those of NNMB survey (35.5%). While there was no such significant differences in CED among females (35.8% vs 38.2%).

Impact of drought on households

- Eleven percent of households reported insufficiency (about 25%) in the consumption of cereals and millets. A higher proportion of HHs reported food insecurity with respect to vegetables (87%), milk & milk products (78.5%), fats & oils (81%) and roots and tubers (84%).
- Coping strategies reportedly adopted by the households during the current drought were availing Government assistance such as TPDS/ AAY (80.2%), consumption of low cost foods (45%), borrowing cash/food from neighbours (38%), disposing household assets (15.5%) and migration (11.8%).

Nutritional status vs socio-economic variables

- No significant differences were observed in the overall prevalence of underweight (<Median -2SD) among children from different communities, HH occupation and landholding.
- Significantly higher proportion of children from marginal farmers were stunted (40.3%) as compared to those belonging to small farmers (10.5%).
- No significant differences in the prevalence of CED was observed between the communities and among households with different extent of land holdings.

2. NUTRITION PROFILE OF INDIANS – A DISTRICT LEVEL SURVEY IN UTTARANCHAL

Investigators : K.Vijayaraghavan, G.N.V.Brahmam, Sharad Kumar, M.Vishnuvardhan Rao, N.Arlappa, K.Venkaiah, A.Laxmaiah, R.Hari Kumar, Ch.Gal Reddy, K.Mallikharjuna Rao, M.Ravindranath, Grace Maria Antony and N.Balakrishna

Duration : 3 years

Date of completion : Dec. 2003

The nutrition profile surveys, for the first time, provided information on diet and nutrition status of communities at the district level. A survey was carried out in the districts of Uttaranchal.

General Objective

The general objective of the survey was to assess the dietary intakes and the nutritional profile of the rural community at the district level in the State of Uttaranchal.

Specific objectives

The specific objectives were :

1. To assess the food and nutrient intake of different segments rural population in the state,
2. To assess the nutritional status of representative segments of population interms of anthropometry and clinical studies, and
3. To assess the knowledge and practices of mothers on breast-feeding, child rearing and socio-cultural aspects of food consumption in relation to health and disease.

Area of study

The survey was carried out during 2001-2002 in all the 9 districts (now divided into13 districts) of the State in collaboration with the Institute of Applied Statistics and Development Studies, Lucknow (IASDS).

Sampling Design

In each district, twenty villages were selected by systematic random sampling procedure coupled with proportion to population size (PPS), giving due representation to all the blocks/taluks. In each selected village, twenty households (HHs) were covered. For this purpose, the village was divided into 5 natural groups, based on streets/*mohalla*/*basti*, of which one group belonged to SC/ST community. From each group, a cluster of 4 contiguous HHs were surveyed, with a random start.

Investigations

The following information was collected by trained investigators from the state using standard procedures and specially prepared pre-tested proformae.

i) Household socio-economic and demographic particulars,

ii) Anthropometric measurements such as height and weight,

iii) Assessment of household food and nutrients intake in every alternate HH, and food and nutrient intake of individuals in a sub-sample of 5 HHs

iv) Frequency of consumption of foods in a sub sample of 10 HHs

v) Prevalence of clinical signs of nutritional deficiency on all the available individuals by using standard techniques, and

vi) Knowledge and practice (K & P) of mothers of preschool children with respect to diet during health and disease, nutritional disorders and utilization of services under intervention programmes in the households covered for diet survey.

RESULTS

Coverage

A total of 3,604 HHs were covered for demographic and household particulars and family diet survey was carried out in 1,717 HHs. Of these, about a half of the households were covered for individual diet survey by 24-hour recall method. Nutritional status, in terms of anthropometry and deficiency signs, was assessed on 12,617 subjects of different age and sex groups.

Food intake

The average intake (per cu/day) of cereals & millets (501 g) and pulses & legumes (49 g) was well above the RDA of 460 g and 40 g respectively. Consumption of protective and income elastic foods such as green leafy vegetables (10 g) milk & milk products (135 g) and sugar & jaggery (25 g) was lower than the recommended level.

Nutrient intakes

The median per CU/day intakes of energy (2393 kcal), protein (70 g), calcium (521 mg), thiamine (2.0 mg) and niacin (19 mg) were higher than the recommended levels which is attributable to high cereal intake, while the intake of micronutrients such as iron (22 mg), vitamin A (135 mg) and riboflavin (0.9 mg) were below the RDA. About 68% of the HHs had adequate dietary energy intakes (^{3} Mean-2SD of requirement), while 95% had adequate intake of protein. Five percent of the households had inadequate intake of both protein and energy. Among the preschool children, except for protein, thiamin and calcium, the mean intakes of all other nutrients were below the recommended levels. The extent of energy deficit was 27% in case of 1-3 year children and 18% in 4-6 years children. In the case of vitamin A, the deficit was relatively much higher in children of 1-3 years (61%) and 4-6 years (60%). Among the younger adolescents (13-15 years), the intake of all the nutrients, except thiamin and niacin, was below the RDI in both the sexes. Similarly, among older adolescents (16-18 years), the extent of deficit was more for iron (56% for boys and 33% for girls) and vitamin A (74% for boys and 71% for girls) compared to other nutrients. In case of adults, except for micronutrients, such as iron, vitamin A, riboflavin and free folic acid, the intake of all other nutrients was above the recommended level. The extent of deficit with regard to mean intake of iron was higher among females (30%) compared to males (14%).

Nutritional Status

The individuals of different age groups were considerably lighter and shorter than the reference population (NCHS). The prevalence of severe undernutrition (<60% weight for age) among preschool children was about 4% and no significant gender differences were observed. Overall prevalence of underweight (weight for age < Median-2SD) was 41%.

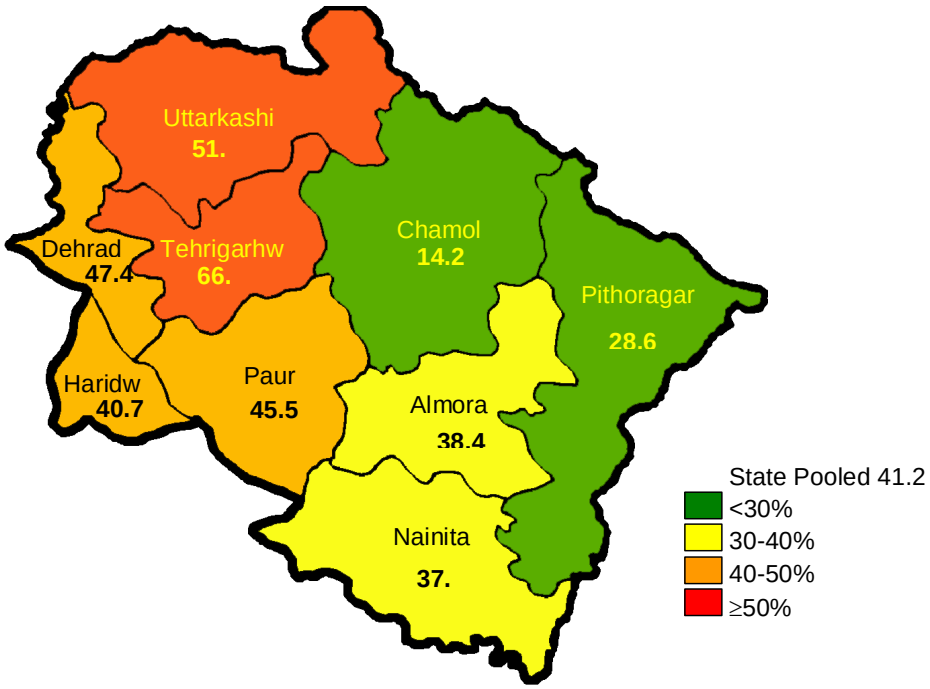
However, the prevalence of under weight though statistically not significant was higher among boys (44.5%) than girls (37.6%). The extent of undernutrition in different districts is presented in the Map (Map.1). The prevalence of stunting (height for age < Median–2 SD) was 64.7%, while wasting (weight for height < Median – 2 SD) was 8.2%.

About 35.7% of males and 29% of the females had different grades of chronic energy deficiency (CED) as measured by BMI (<18.5). About 61.5% of males and 65.5% of the females had normal body mass index (18.5-25.0). The prevalence of obesity (BMI >25) among females was twice (5.5%) that of males (2.8%).

CONCLUSIONS

Thus, the results indicated that the diet and nutrient intakes, especially micronutrients were grossly inadequate compared to RDA. The undernutrition is also widely prevalent in the State, both among the preschool children and adults. The data on diet and nutritional status of population, made available for the first time at the district level, would be useful to the planners to prepare strategies and plan of action to combat malnutrition at district level.

Map 1 showing distribution (%) of Prevalence of underweight (< -2SD weight for age) – among pre-school children in the State of Uttaranchal



3. PREVALENCE OF MICRONUTRIENT DEFICIENCIES - NATIONAL NUTRITION MONITORING BUREAU

Investigators : K.Vijayaraghavan, GNV. Brahmam, A.Laxmaiah, R.Hari Kumar, Arlappa, N., N.Balakrishna, Ch.Gal Reddy, B.Suryaprakasam, K.Mallikharjuna Rao, Sharad Kumar, M.Ravindranath

Duration : 2 years

Date of completion : November 2003

The National Nutrition Monitoring Bureau (NNMB), established under the aegis of the Indian Council of Medical Research (ICMR) has been carrying out regularly, since its inception in 1972, annual surveys on diet and nutritional status of the population in the States of Andhra Pradesh, Gujarat, Karnataka, Kerala, Tamil Nadu, Maharashtra, Madhya Pradesh, Orissa, Uttar Pradesh and West Bengal. The results are published in the form of technical reports. The micronutrient deficiencies such as Iron Deficiency Anaemia (IDA), Vitamin 'A' Deficiency (VAD), and Iodine Deficiency Disorders (IDD) continue to be of public health significance in India. However, there have been no systematic nation-wide surveys representing different States covering a large and statistically adequate sample size to assess the prevalence of micronutrient deficiencies among the vulnerable groups of the rural population. Therefore, NNMB carried out the present survey to assess prevalence of some of the important micronutrient deficiencies among vulnerable groups of rural population covering statistically adequate sample in each of the States where the bureau has been in operation.

Specific Objectives

i) To assess the prevalence of clinical forms of vitamin A deficiency (particularly Bitot Spots) among the pre-school children,

ii) To estimate serum vitamin A levels in preschool children using Dried Blood Spot (DBS) technique,

iii) To estimate haemoglobin levels among preschool children, adolescent girls, pregnant women and lactating mothers,

iv) To assess the clinical prevalence of IDD in the age group of 6-11 years children,

v) To estimate iodine levels in the salt samples collected at household level using spot testing kit and

vi) To assess knowledge and practices of women regarding IDA and VAD and coverage of beneficiaries for the supplementation of IFA tablets and massive dose of vitamin A under national programmes.

METHODOLOGY

Study Design

In each State, 80 villages from 16 strata (districts) [at the rate of 5 villages per stratum], covered for diet and nutrition survey during the year 2000 (NNMB Tech. Rep. No 21) were selected, adopting stratified random sampling procedure, using the sampling frame of 54th round of consumer expenditure survey conducted by the NSSO in the year 1998. The survey was carried out during October 2001 to November 2003 (Table 1).

Investigations

- Assessment of prevalence of clinical signs of vitamin A deficiency, especially Bitot spots among 1-5 year children,
- Estimation of serum vitamin A levels in a sub-sample of preschool children covered for clinical examination, from finger prick blood samples by Dried Blood Spot (DBS) technique, using HPLC method,
- Estimation of haemoglobin levels from finger prick blood samples collected with fixed volume 'Finpipette', by cyanmethaemoglobin method using colorimetry, among preschool children, adolescent girls, pregnant women of ³6 months duration, and lactating mothers of <6 months duration,
- Assessment of prevalence of clinical forms of IDD among 6-11 year children,
- Assessment of iodine in salt samples collected from households using spot testing kits, and
- Assessment of K&P of women on IDA & VAD and their prevention.

Sample Size

Table 1. Criteria used to compute the sample size

Investigations		Age/Sex/ Physiological Group	Estimated Prevalence	C.I	Relative Precision	Sample size per State
VAD	Clinical Exam.	1-5 years	1% (Bitot pots)	95%	20%	9508
	Serum Vit. A	1-5 years	40% (serum Vit A <20μg/dl)	95%	10%	576
IDA	Hb. Estimation	1-5 years	70%	95%	10%	336
		12-14 yrs. Girls	70%	95%	10%	336
		15-17 years Girls	70%	95%	10%	336
		Preg. Women (≥ 6 months)	70%	95%	10%	336
		Lact. Women (<6 months)	70%	95%	10%	336
IDD	Clinical Exam.	6-11 years children	10% (Total Goitre)	95%	10%	3457
	Spot testing of cooking salt for I_2	Households	-	-	-	800
K&P	VAD (Mothers of 1-5 year children)		50^{th} child covered for VAD Clinical			
	IDA (Mothers of 1-5 year children, Preg. & Lact. women)		Alternate individual covered for Hb estimation			

Training of the investigators

All the research staff of different units of the NNMB were standardized at NIN for a period of two weeks in techniques of blood sample collection, estimation of haemoglobin and administration of questionnaires, before the actual survey was initiated. During the training, emphasis was laid on the investigators achieving the maximum intra and inter-observer agreement in respect of clinical signs of VAD, IDD and haemoglobinometry.

Quality Control

Every 10th blood sample drawn for the estimation of haemoglobin was collected in duplicate. While every alternate duplicate sample was analyzed at the NNMB unit itself for intra laboratory consistency, the remaining were sent to NIN-CRL for analysis, to ensure inter-laboratory consistency. Staff from CRL made regular visits to the field in each State to carry out random checks on 5% of the sample covered by the respective NNMB team.

Salient observations of the study are provided below :

Coverage

A total of 75,600 HHs from 633 villages in the States of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Madhya Pradesh, Orissa and West Bengal have been covered for various investigations in the present survey. Survey in the States of Gujarat and Uttar Pradesh had to be dropped due to technical reasons. A total of 71,591 preschool children were covered for clinical assessment for Vitamin A deficiency. While about 3,291 preschool children, 6,616 adolescent girls, 2,983 pregnant women (^{3}24 weeks) and 3,206 lactating mothers (<6 months) were covered for haemoglobin estimation, about 28,440 children of 6-11 year age group were covered for clinical assessment of IDD. K&P on VAD was assessed on 2,681 mothers of index children of 1-5 years of age, while K&P on IDA was assessed on 2,178 mothers of index children of 1-5 years of age, 2,053 pregnant women (^{3} 24 weeks) and 2,213 lactating mothers (<6 months).

Sample Characteristics

About 86% of HHs covered for various investigations belonged to Hindus, while 10% of the sample was Muslims. The proportion of Hindus was lowest in the State of Kerala (55.6%) and high in Andhra Pradesh (95.8%), Madhya Pradesh 95.7% and Orissa (95.4% each).

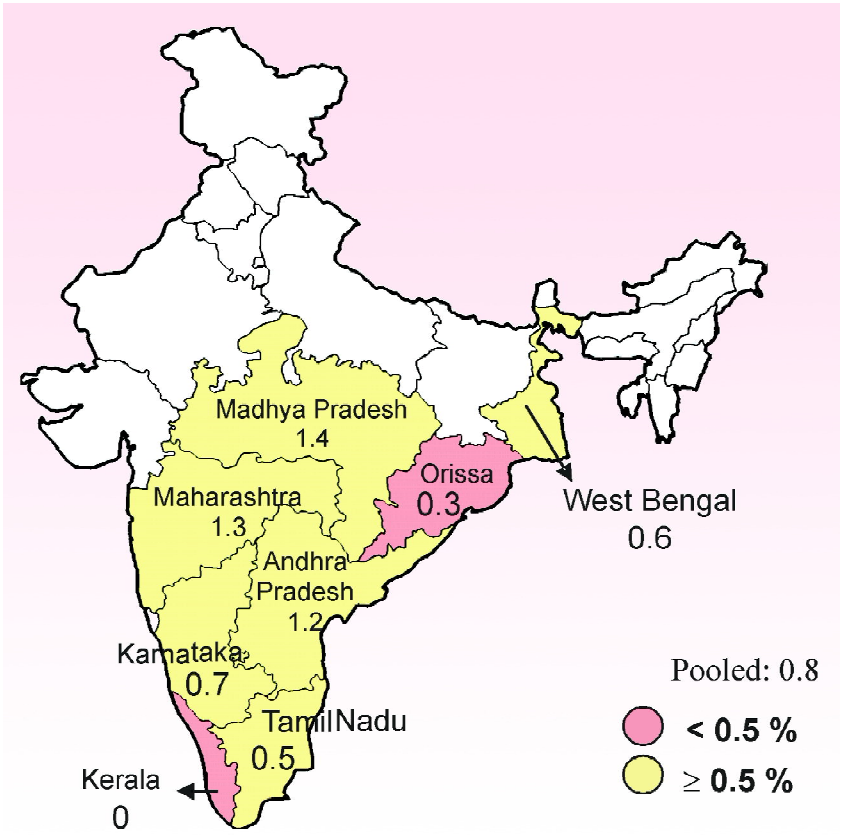
Twenty nine percent of the HHs belonged to Scheduled castes and Scheduled tribes. The proportion of tribals was relatively higher in the States of Madhya Pradesh (27%) and Orissa (17.5%), while the proportion of Scheduled caste HHs was relatively higher in West Bengal (29%), Andhra Pradesh (25.2%) and Tamil Nadu (23.6%) as compared to other States. Labour (non-agricultural labour : 28.7% and agricultural labour :16.5%) was the major occupation in about 45% of the HHs, while about 27% HHs were engaged in cultivation. The overall adult female literacy in the HHs surveyed was about 51%, which ranged from 95% in Kerala to 24% in the State of Madhya Pradesh. The average family size was 4.8.

In general, only about a fourth of the HHs (27%) had sanitary latrines. While a majority of HHs in Kerala (94.3%) had sanitary latrine, their proportion was very low in Madhya Pradesh (8.8%) and Orissa (8.4%).

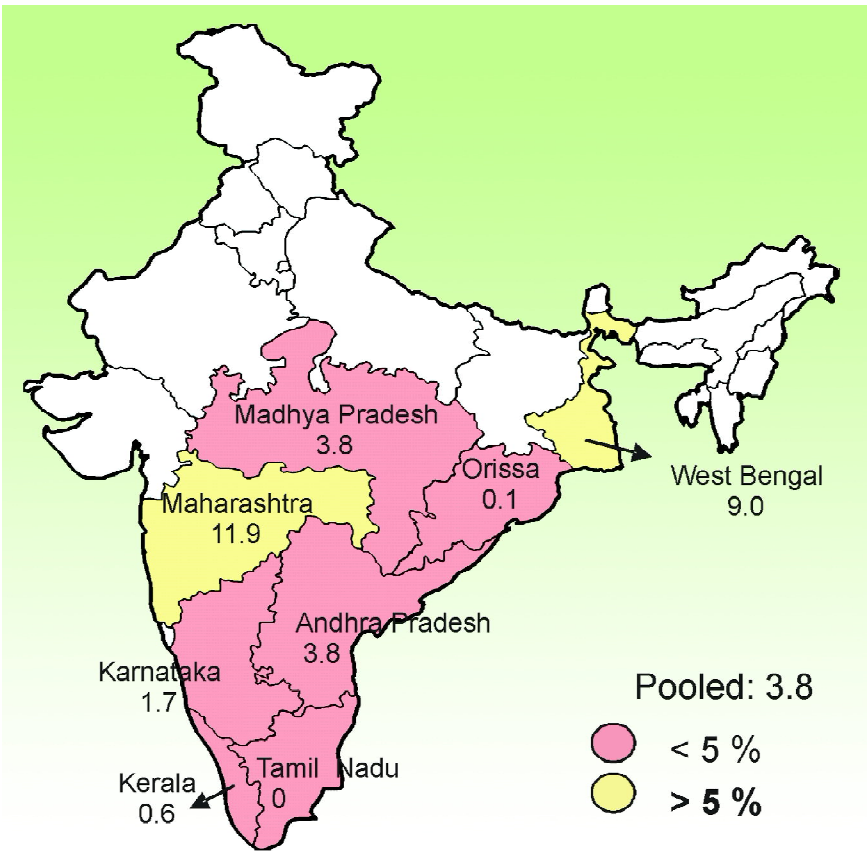
Prevalence of clinical signs of VAD and IDD

The overall prevalence of night blindness among preschool children was 0.3% and that of Bitot spots was 0.8%. The prevalence of bitot spots was >0.5%, a cut-off level recomm ended by WHO to indicate public health significance, in all the States, except Kerala and Orissa (Map 2). The overall prevalence of total goitre among 6 to 11 year children was about 4%, which is below the cut-off to indicate endemicity of IDD. However, the proportion was higher in the State of Maharashtra (11.9%) and West Bengal (9%) (Map 3).

Map 2: Prevalence (%) of Bitot spots among 1-<5 year children



Map 3: Prevalence (%) of IDD among 6 -< 12 year children



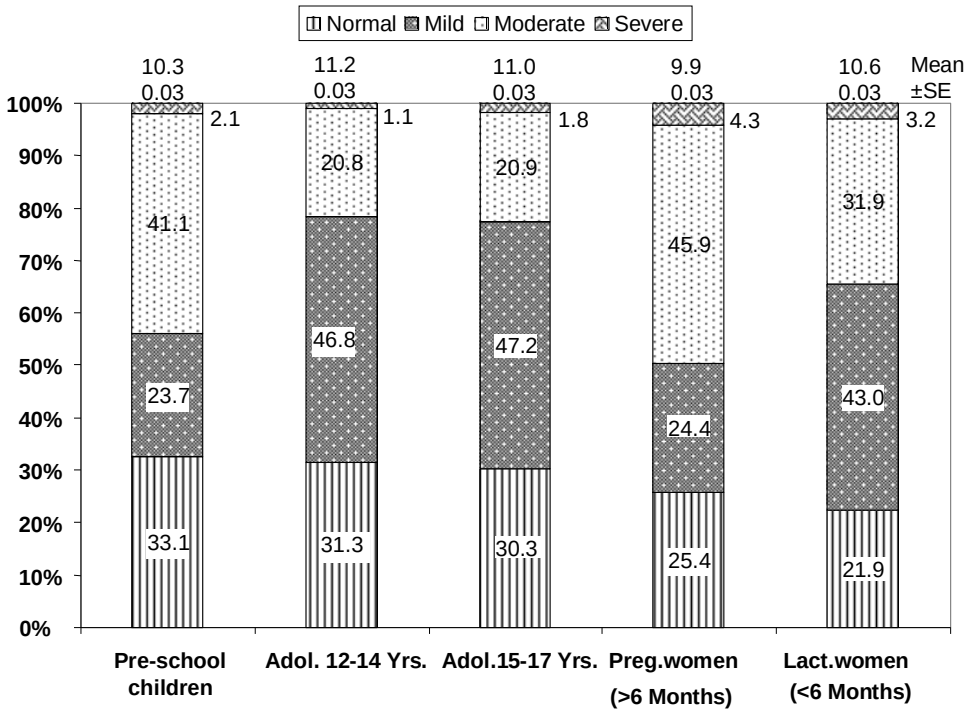
Iodine content in household salt samples

In general, about 42% of the HHs were using non-iodized salt, while in 31% of HHs, the iodine content of the salt was at recommended level of ³15 ppm. In about 27% of the HHs, the iodine content was ≤7 ppm. The proportion of HHs using non-iodized salt was relatively higher in the States of Karnataka (67%) and Maharashtra (58.3%) as compared to others.

Mean haemoglobin levels and Prevalence of anaemia

Mean haemoglobin level among different age/sex/physiological groups ranged from about 9.9 g/dl among pregnant women through 10.3 g/dl in preschool children, 10.6 g/dl in lactating women to about 11 g/dl in adolescent girls. However, the mean haemoglobin levels among all the physiological groups were higher in the States of Kerala as compared to other States. The overall prevalence of anemia among preschool children (<11g/dl) was about 67% while 43% recorded moderate to severe anaemia (<10 g/dl). The prevalence of anaemia was highest in the States of Orissa (92.4%) followed by West Bengal (81.2%), Andhra Pradesh (70.8%) and Madhya Pradesh (64.7%). About 69% of the adolescent girls (12-17 years) were observed to be anaemic, with 22% recorded moderate to severe anemia.

Fig.1: Prevalence (%) of Anaemia among different physiological and Age groups



The prevalence of anaemia among older adolescents (15-17 years) was also highest in the States of West Bengal (87.6%) followed by Orissa (77.6%), Madhya Pradesh (76.1%) and Andhra Pradesh (72.9%). The prevalence of anemia among pregnant women (<11g/dl) was around 75% with 50% recording moderate to severe anaemia. Among the States surveyed, the prevalence of anaemia was maximum in the States of Madhya Pradesh (84%), Orissa (81.5%) and Karnataka (79.8%). The overall prevalence of anemia among lactating women (<12g/dl) was about 78% with 35% having moderate to severe anaemia. The prevalence of anaemia was highest in the State of West Bengal (95.9%) followed by Orissa (91.2%) and Madhya Pradesh (85.9%) as compared to other States.

K&P of women on VAD

Only 42% of the mothers of index children were aware of night blindness. About 8% of the mothers mentioned "permanent blindness" as one of the manifestation of VAD, while about 4% mentioned Bitot spots. About 14% of the mothers attributed it to dietary deficiency, while 12% said that deficiency of vitamin 'A' leads to night blindness. Only about 24% were aware of the role of foods such as green leafy vegetables (18.4%), yellow coloured fruits (11.5%) and animal foods (11.3%) in the prevention of VAD. About 58% of the preschool children reportedly received at least one dose of massive vitamin 'A' (2 lakhs) during the previous one year, while 30% received one dose and about 25% received two doses. About 13% of the mothers reportedly received nutrition education on VAD, and the messages included need for regular consumption of GLV (10%) and yellow coloured foods (6.5%), signs and symptoms of VAD (5.3%); supplementation of massive dose of vitamin 'A' (5.7%) and consequences of severe VAD (4.4%).

K&P of Women on IDA

Awareness of anaemia, in general, was poor among different categories of respondents. Only about 34% of the women were aware of anaemia, the proportion of whom ranged from a high 62.5% in the State of Andhra Pradesh to a very low in Madhya Pradesh (8.5%). About 23% of women reported 'tiredness' as one of the manifestation of anaemia, followed by pallior (17%) and breathlessness (5%). Majority of the respondents attributed anaemia to inadequate diet (26%).

The proportion of beneficiaries who had received IFA tablets during previous 1 year or during pregnancy/lactation was in general, very low among pre-school children (3.8%) and lactating mothers (12.3%). This was relatively higher among pregnant women, which ranged from a low (38.2%) in the State of Kerala to a high (78%) in the State of Maharashtra. However, only about 30% of pregnant women and 2% of the children, in general, reportedly received ^{3}90 IFA tablets. The most common reason for partial receipt of the IFA tablets was that they were 'not offered' (53%), Less than 10% of the beneficiaries reportedly experienced side effects such as nausea, vomiting, passing of black stools etc. Only 14% of women reportedly received health and nutrition education on IDA, and the commonly received messages were on promotion of consumption of iron rich foods (7.9%) and regular consumption of IFA tablets (5.4%).

4. ASSESSMENT OF PREVALENCE OF OVERWEIGHT AND OBESITY IN URBAN ADOLESCENT SCHOOL CHILDREN AGED 12-17 YEARS IN HYDERABAD, ANDHRA PRADESH

Investigators : A.Laxmaiah, V.Mohanan Nair (Achuta Menon Centre for Health Science Studies), N.Balakrishna and K.Vijayaraghavan

Duration : 6 months

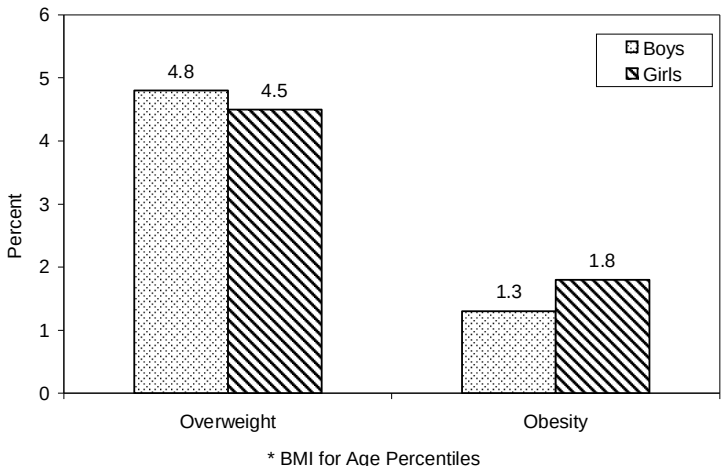
Date of completion : June 2003

Overweight and obesity are emerging as important public health problems in developed as well as developing countries today. The problem is confined not only to adults but also among young children and adolescents. Adolescent period is an important period in the life cycle of human beings, characterized by rapid rate of growth. The most significant long-term consequence of childhood and adolescent obesity is its persistence even during adulthood, with all the associated health risks.

Estimation of prevalence of overweight and obesity and its correlates is, therefore, of paramount importance for the formulation of strategies to avert overweight and obesity. Therefore, a study was undertaken with an objective to estimate the prevalence of overweight & obesity and its correlates among urban adolescent school children (12-17 years) in the city of Hyderabad. Twenty three schools catering to low, middle and upper middle income groups were selected by stratified random sampling procedure. Anthropometric measurements, viz., height (cms) and weight (kgs) were taken on 1,208 adolescent school children using standard procedures. Information on socioeconomic and demographic particulars, their perceptions and practices on diet, life style patterns, physical activities and frequency of consumption of foods was assessed using pre-tested questionnaires. Overweight (^{3}85th - <95 percentile) and obesity (^{3}95 percentile) were defined using BMI for age percentiles of American standards. Stepwise logistic regression analysis was carried out.

The results revealed that in general, the prevalence of overweight and obesity was 6.2%, (Fig.), which was comparable among the girls (6.3%) and boys (6.1%). The prevalence was significantly ($p < 0.001$) higher among children studying in private and private aided institutions (8 and 9%) as compared to those studying in the government institutions (2.4%), among those belonging to upper middle (6.7%) and high socioe-conomic status (13.1%) as compared in the low and low middle socio-economic status (1.7 to 2.5%).

Fig 2. Distribution (%) of adolescents (12-17 Years) according to prevalence of Overweight and Obesity*



It was significantly lower ($p < 0.001$) in the children who were reportedly participating in the household activities for ^{3} 3hrs/day. On the other hand, it was significantly higher ($p < 0.007$) among children (9.3%), who are watching TV for ^{3}3 hrs/day as compared to the children, who are watching for < 3 hrs/day (5%).

Stepwise logistic regression analysis showed that the prevalence of overweight and obesity was 7 times higher among the children, who were watching TV for ^{3}5 hrs/day compared to the children with <5hrs/day, 4.4 times higher in the children who belong to upper middle and high SES compared to the children of low and low middle SES and 3.9 times higher in the children who were studying in public schools compared to those in government schools. Participation in HH activities for ^{3}3hrs/day (OR : 0.3) had some protective effect from overweight and obesity (Table 2).

Table 2. Stepwise Logistic Regression Analysis

Variable	OR	p-value
TV watching > 5hrs/day	7.2 (4-13.1)	0.000
High Socio-economic status	4.4 (2.3-8.7)	0.000
Private & Private aided school Children	3.9 (1.8-8.7)	0.001
Children's interest towards fatty foods	2.0 (1.2-3.6)	0.013
Resident at home	2.0 (1.1-3.7)	0.025
Children's participation in HH activity	0.3 (0.2-0.5)	0.000

Thus, the study revealed that the prevalence of adolescent overweight and obesity among urban adolescent school children in Hyderabad was higher than in their rural counterparts (0.6%). The prevalence was more among the children of upper middle and high SES groups compared to the children of low and low middle SES. The prevalence was relatively less among children participating in physical exercises like games & sports and higher among the children with no physical exercise or who were watching TV for long hours. The prevalence of overweight and obesity was also higher among children who consumed fatty and fried foods and also among those frequently consuming snacks, ice creams etc. There is need to initiate programmes of health and nutrition education for school children belonging to HSE group to encourage physical activity in the form of games and sports.

5. CURRENT STATUS OF IDD IN SELECT DISTRICTS OF DIFFERENT REGIONS OF THE COUNTRY

Investigators : K.Vijayaraghavan, G.N.V.Brahmam, R. Hari Kumar,, M.Vishnuvardhana Rao, K.Madhavan Nair, S.Ranganathan, Ch. Gal Reddy, B. Sivakumar

Duration : 1 Year

Date of completion : December 2003

Iodine deficiency disorders (IDD) are one of the most important micro-nutrient deficiency disorders of public health significance in India, the two major aetiological factors being environmental iodine deficiency and presence of goitrogens in diets. Though, endemic goitre is one of the most common form of manifestation, the iodine deficiency causes a wide spectrum of disorders such as abortion, still births, low birth weight, cretinism, neonatal chemical hypothyroidism, psycho-motor defects, impaired coordination and hypothyroidism. Studies have revealed that in iodine deficient environment, the school children have low IQ and poor scholastic performance. Environmental iodine deficiency is also known to affect the livestock as well, including cattle, sheep, pigs and poultry in the form of abortions, stillbirths, low birth weight, alopecia, inadequate growth, and functional disabilities. With fortification of common salt with iodine, the prevention and control of IDD has become feasible, in terms of both the cost and efficacy.

In India, though classical endemic belt of IDD is known to exist in sub-Himalayan belt, several studies have shown that IDD endemic pockets do exists in peninsular India as well. The data compiled by the Director General of Health Services, Government of India,

from the sample surveys conducted between 1959-99 in 25 States and 4 Union Territories revealed that 239 districts out of 282 districts surveyed were endemic for IDD with the goitre prevalence ranging from 10 - 68%.

Government of India (GOI), in the year 1962, launched National Goitre Control Programme (NGCP). Evaluation of NGCP by the Nutrition Foundation of India in the year 1981, at the request of the GOI, revealed that it did not achieve the desired objectives and several factors responsible for the same were identified. In the light of those observations and in view of the reported wide spread problem of IDD, GOI in 1984, launched the programme of Universal Iodization of salt, with an objective to iodize entire edible salt in the country in a phased manner, by 1990. In 1988, PFA Act was amended to specify that iodized salt should have iodine in the concentration of 30 ppm at production level and 15 ppm at consumer level.

Recently, in spite of protests from the medical and scientific community, the Government of India, vide in its Gazette notification (No. G.S.R. 716 [E], dt. 13th September 2000) lifted the ban under the PFA on sale of uniodized salt. Thus it was felt necessary to assess the current status of IDD in different regions of the country. This is expected to help the policy makers to adopt appropriate strategy for prevention and control of IDD.

Hence, at the request of DGHS, Government of India, the National Institute of Nutrition, along with four collaborating institutes carried out IDD surveys in select districts of different parts of the country.

General Objective

The general objective of the study was to assess the prevalence of IDD in a representative sample of the districts in different regions of the country, that were surveyed between 1959 and 2000.

Specific Objectives

The specific objectives of the study were:

- To assess the prevalence of clinical forms of IDD among 6-12 year children in selected district of the States in different regions in the country,
- To assess random urinary iodine excretion levels in a sub sample of 6-12 year children covered for clinical examination, and
- To assess the use of iodized salt by the households by spot test for iodine.

METHODOLOGY

Study Design

The study was carried out in a sub-sample of 10% of the districts that were surveyed earlier, representing different States/regions in the country, with maximum prevalence of goiter. For this purpose, the State wise list of districts with goiter prevalence figures provided by DGHS were utilized. In each of the selected district, IDD survey was carried out by using 30 cluster sampling method.

Sample size

In each of the district to be surveyed, presuming an anticipated goiter prevalence of 10%, for a 10% relative precision of estimate, at 95% CI, a sample size of 3,457 children in

6-12 year age group was arrived at. Since it is proposed to carry out the survey by *'30 cluster sample'* method, considering the design effect of '3', the total sample size to be covered was calculated as 10,371 or say 10,500 per district or 350 children per cluster.

Similarly, owing to salt iodization programme, the median urinary iodine excretion levels in a community are expected to be normal (^{3} 10 mg/dl). Hence, with an estimated prevalence of 50% normal (^{3} 100 mg/dL), a relative precision of 80% and design effect of 2, a sample size of 200 children was arrived at for district for estimation of the urinary iodine levels.

Investigations

The following investigations were carried out:

- Clinical Examination of 6-12 year children for presence of clinical forms of IDD.
- Estimation of Urinary iodine excretion levels from random urine samples collected from a sub sample of children covered for clinical examination by wet digestion method.
- Testing of Household Salt samples for Iodine content , both by spot testing kits and volumetric method.
- Knowledge and practices of women about IDD and use of iodized salt, and
- Extent of sale of iodized salt in the local shops.

Collaborating Centres

For the purpose of survey, the country was divided into five geographical regions viz,., Northern, Northeastern, Eastern, Central and Southern regions. In each region, a total of 8 districts with maximum prevalence of goiter were selected (@ of 1-2 districts per State). The investigating centres were, Department of community medicine, PGIMER, Chandigarh (Northern Region), Department of S.P.M. Assam Medical College, Dibrugarh (North Eastern Region), Department of S.P.M., Government Medical College, Surat (Central Region), Regional Medical Research Centre, Bhubaneswar (Eastern Region) and National Institute of Nutrition, Hyderabad (Southern Region). All the participating centers followed a uniform protocol and all the investigators were trained and standardized at NIN for ensuring quality control of data. NIN, in addition coordinated the survey study in all other regions, in the country.

The collaborating center of central region, due to logistic reasons, could cover only 3 districts, while the remaining were covered by Northern Region (3 districts) and Southern Region (2 districts).

Training of the Investigators

The Senior investigators/Research assistants of all the regions were trained and standardized in the techniques of sampling, administration of questionnaires, clinical examination of individuals for assessing IDD, testing of salt for iodine, collection/storage/ transportation of urine samples, estimation of urinary iodine levels and data entry.

Quality Control

Senior staff of the collaborating centers conducted random checks of 10% of data collected in the field by the investigators, to ensure quality. In addition duplicate samples of salt and urinary samples were collected and analyzed by the respective centers to maintain intra-investigator consistency. Also a set of duplicate samples was sent to NIN for ensuring inter-laboratory consistency.

RESULTS

Coverage

The coverage in most of the districts satisfied required sample size, except for the districts of Chandel in Manipur, Changlong in Arunachal Pradesh, Shimla, Kullu in Himachal Pradesh and Gurudaspur in Punjab, where the coverage for clinical examination was less by 1-10% due to logistic reasons. Similar low coverage was also observed for titrimetric analysis of salt samples in the districts of Kolhapur and Bikaner by 8-10% and analysis of urine sample for estimation of iodine excretion by 36% in the district of Saharanpur of Uttar Pradesh.

Salient features of the results are summarized as follows:

* The overall prevalence of total goitre has declined significantly from about 14% - 69% during 1984–94 to about 3% to 40% in the districts surveyed.

* In the northeastern region, the current prevalence of total goiter ranged from 5 to 9% in all the districts surveyed, which is less than the cut-off level of 10% recommended by the Govt. of India to indicate endemicity of IDD. In contrast, in the eastern region, the prevalence of goiter either remained same or increased in three districts, while in the remaining district, the extent of decline was relatively less.

* Majority of the goiter cases reported were of Grade I, while that of Grade II were negligible.

* The prevalence of other signs of IDD such as deaf-mutism, mental retardation and squint was <1% and that of cretinism was negligible in all the districts surveyed.

* The median urinary excretion level was <100 mg/L in 9 out of 40 districts surveyed, of which 4 were from central region, 3 from northern region and one each from eastern and southern regions.

* In 14 out of 40 districts, the urinary iodine excretion levels were <50mg/L in ^{3}20% of children examined, indicating, endemicity of IDD. Of these districts, 5 were from central, 4 from eastern, 3 from northern and one each from northeastern & southern regions.

* The proportion of households consuming adequately iodised salt (iodine ^{3}15 ppm) as assessed by the spot test, was observed to be maximum in the Northeastern (64 to 89%) and Northern region (barring two districts) (60-88%). In very few districts in the remaining regions, >60% of HHs were found consuming adequately iodized salt.

* Volumetric analysis of salt samples revealed that the proportion of HHs consuming uniodised salt in different districts was observed to be lowest in northeastern (nil) and eastern region (nil to 0.5%), followed by northern and southern regions (nil to 7%) and central region (2 to 32%).

* In most of the districts surveyed, in all the regions except northeast, the quality of iodized salt was unsatisfactory and the iodine content ranged from 7-15 ppm in majority of the salt samples analysed.

* The awareness of the women about the iodized salt was observed to be maximum in the Northeastern region (30 to 58%), followed by eastern (10 to 33%), central (10 to16%) and Northern & Southern regions (<10%).

* Only a negligible proportion of women were aware of ‘smiling sun’ as logo of iodized salt.

6. DIFFERENCES IN ATTENTION-CONCENTRATION, MEMORY AND SCHOOL ACHIEVEMENT OF REGULAR AND IRREGULAR BREAKFAST EATERS AND NON-EATERS

Investigators : Shahnaz Vazir, Nitin Gajre, N. Balakrishna

Duration : 6 months

Date of completion : December 2003

Background

The potential role of the consumption of breakfast in helping children and adolescents perform better in the classroom was noticed more than 30 years ago by educators. Irregular eating patterns are common during late childhood and adolescence. Breakfast appears to be the most frequently missed meal due to various reasons.

Not eating breakfast has been associated with overall inadequate nutrient intake (Morgan, 1986). The neuro-muscular system runs on glucose, the fuel, needed to carry out both, mental and physical activities. Skipping breakfast starves the body of energy for at least 10-12 hours. The brain, therefore, remains deprived of energy until the body breaks down the stored carbohydrate, fat and protein. For children, omission of breakfast may show up in poor school performance.

A cross-sectional pilot study was, therefore, undertaken to investigate the relationship between breakfast consumption vis-à-vis attention-concentration, immediate recall memory and academic achievement among school children aged 11-14 years from middle class urban families.

Specific Objectives

1. To determine the routine eating habits of school children with specific reference to consumption of breakfast.
2. To assess the attention- concentration & immediate recall memory of the children by administrating culture appropriate tests.
3. To assess their nutritional status by measuring their height and weight and
4. To compare the school marks/ grades obtained by the study children during the previous year.

Study design and sample

The study was carried out in two schools catering to middle class population in Hyderabad. All children in the age group of 11 to 14 years, studying in 6th, 7th and 8th classes were included.

Based on the responses of the children to the questionnaire on breakfast habits, they were categorized into three groups, viz., regular breakfast consumers, irregular eaters (2-3 times/week) and non-breakfast-eaters. Breakfast was defined as " the first meal of the day that was taken in the morning, before going to school", which include solids and/or liquids excluding caffeine drinks.

Assessments

The following tests were administered to all the children before categorizing them into three groups.

1. Letter Cancellation Test was administered to the students in each class as a group test during the morning session of school between 9 to 11 am to assess attention-concentration.
2. Immediate Recall Memory Test was also administered to the subjects individually during the first morning session of the school.
3. Heights and weights were measured using standard techniques.
4. Information on socio-economic status such as education level and occupation of parents, community, type of family, house ownership, family assets was collected.
5. The annual marks obtained by each student in the subjects of English, Science and Maths during the previous year were also collected from the school records.

Data Analysis

The data was analysed using appropriate statistical tests such as Chi-Square, ANOVA with Post-Hoc test and Multiple regression. Statistical package for Social Science (SPSS) version 11.5 was used for the data analysis.

The salient observations of the study are as follows:

* Of the 379 children studied, 55.1% were boys and 44.9% were girls. About 44% subjects had completed 11 years of age, 38.5% completed 12 years of age and 17.2% had completed 13 years of age.
* About 62.3 % of subjects regularly consumed breakfast every morning before coming to school, 33.8 % were irregular in taking breakfast as they skipped it 2 or 3 times a week. Only 3.9 % took no breakfast at all and they were classified, as absolute breakfast skippers (No breakfast group).
* A majority of the students (71.6%) belonged to nuclear type of family, while the rest belonged to joint or extended nuclear family.
* It was found that 90-95% of the children in different groups had normal weight for age and about 87% had normal height for age. The nutritional status (weight and height for age) was not observed to be significantly associated with the breakfast habit of the subjects.
* Regular Breakfast Group achieved significantly ($p<0.05$) higher scores (145.3) on the letter cancellation test compared to the 'No Breakfast group' (124.8). These results suggest that those subjects who regularly consumed breakfast performed significantly better on a test of attention-concentration compared to those subjects who skipped breakfast regularly. Results of the multiple regression analysis indicated significant positive association between immediate recall memory and regular breakfast eating habit.
* The 'Regular' group achieved significantly ($p<0.05$) higher over all aggregate marks (63.3%) compared to 'No Breakfast' groups (52.2%). Thus, it is clear from results of the present study that, eating breakfast on regular basis has beneficial effect on the school performance of the children.

* There was a significant ($p<0.05$) positive association between the Letter Cancellation score (attention-concentration) and the regular breakfast eating habit of the children.

* The immediate recall memory test was significantly ($p<0.001$) positively associated with regular breakfast eating habit as well as the weight for age. Students who were regular breakfast eaters and were better-nourished (weight for age index) achieved higher scores on the immediate memory test.

* Significant association ($p<0.01$) was found between the marks obtained in Mathematics during the previous year and the maternal occupation. However, mothers' occupation (Government or Private service) explained only about 2.5 percent variation.

* Nuclear type of family and regular breakfast habit were significantly ($p<0.001$) positively associated. Height for age was negatively associated with school marks obtained in Science. These variables explained about 6.2 percent variation. The negative association of height for age in the present study, however, could not be explained except due to the small sample size.

* Education of the mother (Graduate and above) and Regular Breakfast habit were significantly positively associated with the marks obtained in English, explaining 4.8 percent variation.

* The aggregate marks obtained by the students during the previous year, was significantly ($p<0.01$) positively associated with the Regular Breakfast habit but explained only 1.9 percent variation.

Breakfast consumption was an important factor affecting cognitive functions especially attention-concentration and immediate recall memory of the students particularly during the morning session of the school. However, regular consumption of breakfast *per se* had no significant impact on the nutritional status of the subjects. Eating breakfast regularly was a significant factor influencing better academic performance with higher marks obtained by them.

The sample size, particularly in the 'no breakfast' group is too small and therefore the results of the study can be viewed more in the light of providing indicators for building hypothesis for future work on this topic.

7. INTEGRATED TRAINING AND EDUCATION THROUGH 'AAA' FOR REDUCTION OF MALNUTRITION IN 5 STATES

Investigators : K.Vijayaraghavan, G.N.V.Brahmam, K.Mallikharjuna Rao, Sharad Kumar, M.Ravindranath, M.Vishnu Vardhana Rao and D.Hanumantha Rao

Duration : 2 years

Date of completion : January 2000

The National Nutrition Policy (NNP) envisaged establishment of a Nutrition Surveillance System (NSS) in the country by the Department of Women and Child Development (DWCD) Government of India and identified National Institute of Nutrition (NIN) and National Nutrition Monitoring Bureau (NNMB) to develop it. The NIN developed and tested the feasibility of the system in Andhra Pradesh during 1996-1998 using ICDS infrastructure by

adopting triple A (assessment, analysis and action) approach for early identification of undernutrition, analyse the causes and initiate appropriate action, so as to promote optimal nutrition, right from the anganwadi level (Ann. Rep. 1996-98).

A National Workshop organised at NIN in 1998, reviewed the results of the study in Andhra Pradesh and recommended that the surveillance system should be extended to other States with necessary modifications in respective states. The DWCD, GOI, therefore, requested NIN to establish NSS in the States of Maharashtra, Rajasthan, Karnataka, Madhya Pradesh and Meghalaya. Hence, NIN initiated the NSS in these States.

Work done during the year

Initially joint workshops were organized at the State and Divisional level in the five States for the officials of ICDS and Health Department. Subsequently, the training of trainers (TOT), ICDS and health functionaries in all the five States was carried out (Annual Report 2001-2002), who in turn train the remaining staff in NSS in their respective areas.

The data collection using modified quarterly progress reports was initiated in the States of Meghalaya and Karnataka. So far three quarterly review meetings (June 02, September 2002 and September 2003) in Meghalaya and two quarterly review meetings in Karnataka (September 2002, December 2003) were conducted. During these meetings, NIN investigators reviewed filled in formats along with the respective local staff using specific checklists/ guidelines to assess the consistency and quality of reporting for various indicators. In addition, validation of AWWs data in the State of Meghalaya was carried out to assess the accuracy and quality in data collection.

The salient observations for the State of Meghalaya are summarized below:

- The extent of submission of surveillance reports was comparatively better by Anganwadi Workers (98.9%) than other functionaries, viz., Supervisors and CDPOs (80-85%) during the first quarter. Reporting by the other functionaries, however, improved during subsequent quarters.
- The quality of reporting by ICDS functionaries on various parameters such as enumeration of population, grading status of children nutrition, supplementary feeding, coverage of beneficiaries in various national nutrition programmes, improved gradually from first quarter to the fourth quarter. However, reporting with respect to identification of growth faltering, nutritional deficiency signs, incidence of morbidities, recording of birth weights was not satisfactory even by 4th round. They needed further improvement.
- In general, the extent of application of AAA approach by anganwadi workers over the quarterly periods showed improvement from 60-70% in the first quarter to about 80-95% in the last quarter for different process indicators such as supplementary feeding, morbidity coverage for vitamin A and IFA distribution programs etc.
- Verification of data collected by AWWs by NIN investigators in the field revealed that the information on enumeration of beneficiaries, recording of births and deaths, age assessment of children, plotting of weight and nutrition grading of children on growth charts was being done correctly by a majority of the workers. However, about 50% of the workers did mistakes in compiling of data on nutritional grades according to age and sex. Similarly, in about 15% of cases, the cause of mortality was not reported correctly indicating the need for constant supervision and further orientation of staff in implementation of NSS.

8. PREPARATION OF A DRAFT COUNTRY INVESTMENT PLAN FOR NATIONAL FOOD FORTIFICATION EFFORTS FOR INDIA

Investigator : B. Sivakumar, K. Madhavan Nair, GNV Brahmam and K. Vijayaraghavan

Duration : 6 months

Date of completion :

BRIEF OUTLINE

Considering the long-term implications of micronutrient deficiencies in the population, there is an urgent need to effectively implement programs aimed at controlling them. A situation analysis existing in the country therefore becomes essential. The components required for effective food fortification program need to be identified. Such an exercise is expected to define the priorities in food fortification needs of the country. A comprehensive implementable CIP can then be formulated.

Hypothesis

The hypothesis is micronutrient fortification of supplementary food under ICDS programme will lead to an improvement in growth, development and overall heath and well being of child beneficiaries.

AIMS AND OBJECTIVES

To prepare a draft Country Investment Plan (CIP) for National food fortification efforts for India.

The specific objectives are:

- To identify various supplementary foods being distributed under the ICDS programme for micronutrient fortification
- To develop suitable technology of fortification of the above supplement with micronutrients
- Operationalization of fortification of supplementary foods in the selected areas
- To monitor the program implementation and evaluate its impact on growth, development, micronutrient status and incidence of morbidity among the child beneficiaries

RESULTS

The micronutrients identified for inclusion in the supplementary food are iron, iodine, zinc, vitamin A, thiamin, riboflavin, folic acid, ascorbic acid. The proposal envisages introduction of CIP in the states of Andra Pradesh and Rajasthan for 3 years and the investment required. The CIP is also being submitted to various Government agencies for their comments.

A draft proposal on “Country investment plan (CIP) for the provision of micronutrient fortified food supplement to 0-6 year children through integrated child development scheme (ICDS) in two states” was prepared and submitted to The Keystone Center.

II. NUTRITION AND INFECTION

1. STUDY OF LACTATION RELATED CHANGES IN BONE MASS IN WOMEN FROM LOW SOCIO-ECONOMIC GROUP

Investigators : Bharati Kulkarni, Veena Sh\atrugna, Jessy Metalda, P.Ajeya Kumar, Usha Rani, A.N.Naidu, Balakrishna, N.

Duration : 2 years

Date of completion :

BRIEF OUTLINE

Breast feeding is associated with transfer of approximately 200mg/day of calcium from mother to the infant. Earlier studies have demonstrated that this increased calcium demand leads to mobilisation of this important mineral from the mother's skeleton, leading to transient reduction in bone mineral density (BMD) of lumbar spine and femoral neck regions (4-7%) during 3-6 months of lactation (Sowers et al., JAMA, 1993; 26g:3130-5, Affinito et al., J Clin Endocrinol Metab 1996; 81:2314-8).

Complete restoration of bone density after weaning has been reported in some studies.

The subjects in the above studies had high dietary intake of calcium (>1gm/day) and shorter duration of lactation (<6 months). On the contrary, poor Indian women subsist on a diet with inadequate calories, proteins as well as calcium and they continue to breast feed for a prolonged period of time (>1year). It is possible that the calcium from their diet is inadequate to allow bone-accretion during the recovery period. This study was therefore, initiated to investigate whether lactating women from low socio-economic group lose bone mass during postpartum period and whether low calcium intakes allow restoration of bone density.

This study was approved by the Institutional Ethical Committee.

METHODOLOGY

Sample size

Since there was no available data from India, sample size was arrived at based on initial and six months BMD values generated in a small number of women. The sample size required worked out to be 40.

Fifty postpartum tubectomised women who came to Nutrition Unit in Government Maternity Hospital for their first check-up after delivery were recruited for this study. Their baseline anthropometry and bone-density was studied by DEXA (4500 W, Hologic) at lumbar spine (LS), Hip including femoral neck (FN), forearm and Whole body Bone Mineral Content (WBBMC) within one month after delivery. Fasting blood sample was collected for biochemical investigations like Hb, serum calcium, serum phosphorus, serum proteins, serum total and bone specific alkaline phosphatase and serum total and bone specific acid phosphatase. Breast milk sample was collected for estimation of calcium. Dietary intake

was estimated in a sub sample of women at 2 time points by 24 hrs recall method. First within 6 months after delivery and then at around 1 1/2 years after delivery.

These subjects were followed-up and their bone density measurement and biochemical parameters were repeated at 6 months, 1 year, and 1½ years.

Work done during the year

Out of the fifty recruited women who had DEXA measurement at baseline, 26 subjects have completed the 1½ years follow-up. Another 15 women are expected to complete their follow-up by June 2004.

Thus, in 26 subjects the bone density measurements were repeated at all four time points i.e. baseline, 6 months, 1 year, and 1½ years after delivery and the results are as follows.

1. *Characteristics of the study group at baseline (Mean ±SD) (n=26)*

Age	-	23.4 ± 3.75 yrs
Parity	-	2.6 ± 0.79
Weight	-	46.3 ± 5.39 kg
Height	-	150.7 ± 4.77cm
BMI	-	20.4 ± 2.65

The anthropometric parameters correspond with those reported by the NNMB and are thus representative of the low socio-economic group.

1. *Duration of breast feeding and return of menstruation:* Twenty three out of twenty six women were breast feeding their children even at 1½ years after delivery. Two women stopped after 6 months and one woman stopped feeding after one year.

2, Resumption of menstruation was at 6.7±4.83 months post partum. Dietary intake of all the nutrients including energy (1900±460 Kcal/day), proteins (43±12 g/day) and calcium (448±223.7 mg/day) were below RDA and were not different at the two time points. They were also less than that reported by the NNMB survey.

3. *Changes in Bone density parameters during lactation:* The BMD of the hip, spine and forearm and WBBMC were much lower than those reported from the Western studies in the similar age groups. But they are similar to those reported for the low socio-economic group from this institute (Ann.Rep.2002).

i) BMD at femoral neck - At 6 months after delivery, there was a 4.5% (±5.52) ($P<0.005$) loss of the BMD and it was maintained till one year after delivery. There was only a partial recovery of BMD at 18 months but it was still lower by 2.2% (±5.53) from baseline ($P<0.05$).

ii) BMD at lumbar spine (LS-BMD) – There was no loss of BMD due to lactation at 6 months after delivery and BMD increased significantly later on at one year and it was 3.1% (±5.17) ($P<0.05$) more than the baseline value. The upward trend was observed even at 18 months, as BMD was 5.8% (± 5.66) ($P<0.001$) more than at the baseline.

iii) BMD at forearm_- At 6 months after delivery, there was no loss of BMD. But it decreased later and at 1 year, there was a deficit of 2.4% (±3.16) ($P<0.05$) from the baseline. But the BMD showed complete recovery by 1½ years.

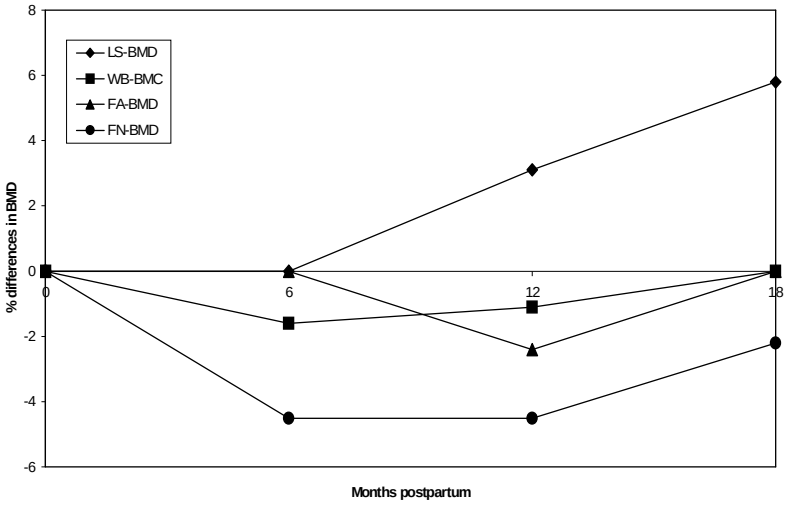
iv) WB-BMC - There was a loss of WB-BMC [1.6% (±3.2) (P<0.05)] at 6 months. But value at one year showed an increasing trend and at one and half year, recovery was complete.

It is observed that the 4 sites exhibit different patterns of change during lactation, probably because of the difference in the cortical and trabecular bone with FN having mainly cortical, LS mainly trabecular (which has a high remodelling rate) and forearm, a mixture of the two. In addition, these women had not reached their peak bone mass which may explain the continued gain of LS-BMD after 6 months inspite of prolonged breast-feeding. Even at the FN site, opposing effects of calcium drain for lactation and accrual of peak bone mass resulted in a much smaller loss than that expected due to low calcium intakes.

There was a wide variation in the pattern of BMD changes. Hence the women were divided into two groups to see if the women who gained BMD at 6 months (LS-BMD) were younger (and had not achieved PBM) than those who lost BMD.

First group included those who lost LS-BMD at 6 months (n=18) (mean loss of 3.8±2.04%) and the second group included those who gained LS-BMD at 6 months (n=9) (mean gain of 3.2±4.79%). When these two groups were compared, there were no differences seen in their mean age, height and baseline LS-BMD. But there were significant differences in their body weights as well as BMI, with the second group having better nutritional status than the first one (Weight – 49.4±6.76 Vs 44.7±3.89 kg) (P<0.05) and (BMI –21.9±3.07 Vs 19.6±2.05) (P<0.05). This reflects the role of nutrition.

Fig 3. Changes in BMD at 4 skeletal sites during lactation



Resumption of menstruation was also reported to be earlier in the second group than the first one (4.2±4.41mths vs 8.0±4.6mths) (P=0.05). Among biochemical parameters, both the markers of bone formation (Bone specific alkaline phosphatase) and bone resorption (Bone specific acid phosphatase) did not show significant change during lactation but a trend was seen for the decrease in the level of bone specific acid phosphatase at 6 months (7.5±1.29 IU/L at baseline Vs 5.9±3.74 IU/L at 6 months) (P=0.07). Breast milk calcium of these women was 218±73.2mg/L, which is similar to the reported values. Inspite

of low intake and prolonged drain of calcium through breast milk, LS-BMD continued to rise in better-nourished mothers. It is possible that conservation of calcium occurred through either increased absorption or reduced excretion or both. But these compensatory mechanisms could offset the breast milk calcium loss only in mothers with better nutritional status.

Thus it can be speculated that, at the time of building maternal peak bone mass, in spite of low calcium intake, the negative effect of lactation may be spontaneously compensated provided the mothers have better body weights and BMI's. The link between bodyweight and BMI with peak bone mass would be further explored in future studies.

III. MICRONUTRIENTS & TRACE ELEMENTS

1. IRON AND ZINC INTERACTIONS AT THE SITE OF ABSORPTION IN RATS

Investigators : B. Sreedhar and K. Madhavan Nair

Duration : 5 years

Date of completion : December 2003

BACKGROUND

Both iron and zinc deficiencies co-exist in certain vulnerable segments of the population, especially pregnant women and children. During their deficiency, supplementation of these nutrients either singly or together is inevitable. In view of their similar physico-chemical properties and scanty reports on their potential interaction during absorption, it is important to understand their consequential effects. The aim of the work was to understand the interactive effects of iron and zinc at the site of absorption.

The effects of iron and zinc supplementation in iron-deficient rats has been reported earlier *(Annual Report 2001-2002)*. It was observed that concurrent supplementation of iron and zinc during iron-deficiency though negatively affected the iron and zinc status, protected the gastrointestinal tract of rat against oxidative stress. The effects of iron and zinc supplementation in zinc deficient and iron & zinc deficient combined deficiency, rats were carried out during this year (Biochem Biophys Res Commun 318.992-997, 2004).

METHODOLOGY

Study design

The study design followed for both the sets of experiments were essentially similar to that adopted earlier. The only difference being that the rats depleted of both iron and zinc were depleted with half the daily dose used in repleting either the iron or zinc deficient rats. The design of the study is given in Figure -4

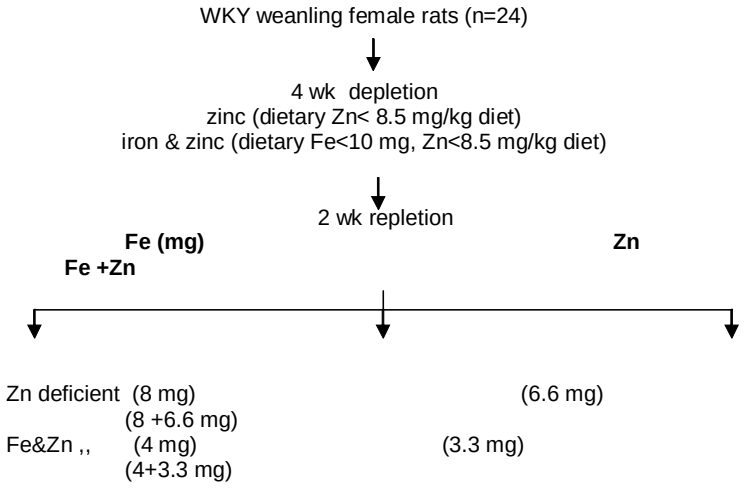


Figure 1: Study design

Absorption of iron and zinc

On the first day of repletion, a set (n=3) of rats received trace amounts (37 mBq) of either ^{55}Fe and/or ^{65}Zn, along with the oral dose. Blood was drawn after 1, 2 and 3h to assess the plasma radioactivity.

Iron and Zinc status

At the end of 2 wk, blood, duodenum and liver were collected. Hemoglobin, serum and liver iron, serum and intestinal ferritin and transferrin and intestinal cytosolic aconitase activity (100000 g) and mitochondrial aconitase activity (12000g)) serum and liver zinc, along with serum and intestinal mucosal metallothionein were measured.

Functional integrity of duodenum

Activity of marker enzymes, alkaline phosphatase and lys, ala-dipeptidyl amino-peptidase (12000g) were measured.

Markers of oxidative stress

Oxidative damage at the site of absorption was determined by measuring the thiobarbituric acid - reactive substances (TBARS) and protein carbonyls in the intestinal mucosa.

Antioxidant status

Activity of antioxidant enzymes in the intestine, namely Cu, Zn-SOD (100000g), Mn-SOD (12000g), catalase (12000g) and glutathione peroxidase (100000g) and non-enzymatic antioxidants such as intestinal reduced glutathione, serum ceruloplasmin (ferroxidase activity) and a-tocopherol concentrations were also determined.

Statistical analysis

Statistical analysis was done by one-way ANOVA followed by post-hoc multiple comparison 't' test. The indicators of deficiency of both iron and zinc were compared with WKY littermates maintained in the colony.

RESULTS

The salient observations of both the experiments are presented together.

1. Zinc deficiency and iron and zinc (combined) deficiencies lowered hemoglobin and serum zinc levels.

2. Repletion of zinc deficient rats with iron and zinc together reduced the AUC of plasma radioactivity of both ^{55}Fe (1.88) and ^{65}Zn (1.25 fold) compared to individually supplemented groups. While the extent of reduction in absorption in iron and zinc deficient rat was 3.7 (^{55}Fe) and 3.5 (^{65}Zn) fold when repleted with both the nutrients together.

3. There was no effect on iron status when supplemented with zinc while iron alone improved in zinc deficient rats. Indicators of both iron and zinc status lowered both in zinc and combined deficient rats when repleted with +Fe+Zn together (Figure - 5).

4. Concentration of TBARS and protein carbonyl was highest in the intestine of both zinc and iron+zinc deficient rat repleted with +Fe and lowest with +Zn (Figure -6).

5. Functional integrity was lower in +Fe and significantly higher when repleted with zinc alone or iron and zinc together.

Cytosolic and mitochondrial aconitase enzyme activities were higher in +Fe, and lower in +Fe+Zn. Among all the groups, +Zn showed the lowest activity (Figure- 6).

Fig 5. Iron and zinc status in zinc and combined deficient rats supplemented with zinc, iron and zinc+iron

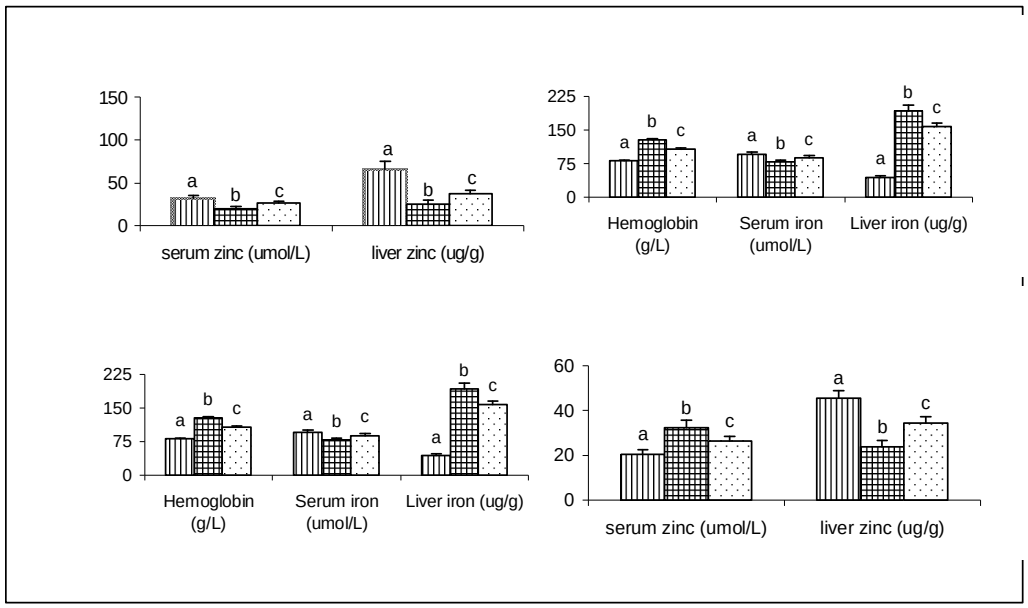


Figure 5. Iron and zinc status indicators in zinc deficient (left panel) and combined deficient (right panel) rats supplemented with zinc, iron and zinc+iron. Vertical bars with different superscripts are significantly different at P<0.05. The dose of iron and zinc used for repletion in zinc deficiency was 8 mg iron and 6.6 mg zinc where as for combined deficiency 4 mg iron and 3.3 mg zinc.

Fig 6. Intestinal oxidative damage indices and aconitase activity in zinc and combined deficient rats

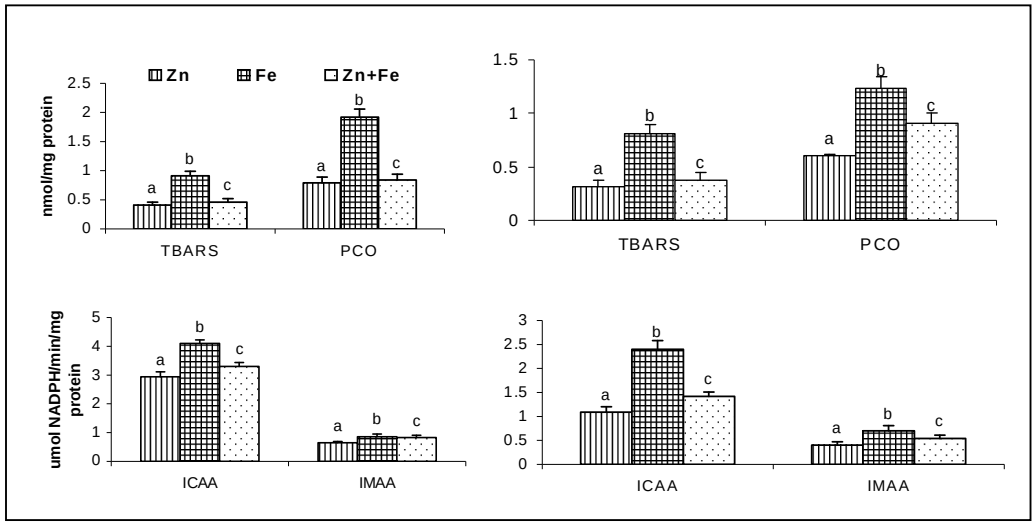


Figure 6. Oxidative stress and aconitase activities in the intestinal mucosa of zinc (left panel) and combined deficient (right panel) rats repleted with zinc, iron and both together. Vertical bars with different superscripts are significantly different at P<0.05. TBARS-thiobarbituric acid reactive substances; PCO - protein carbonyls; ICAA- intestinal cytosolic aconitase activity and IMAA - intestinal mitochondrial aconitase activity. The dose of iron and zinc used for repletion in zinc deficiency was 8 mg iron and 6.6 mg zinc where as for combined deficiency 4 mg iron and 3.3 mg zinc.

1. SOD, catalase, glutathione peroxidase activities were significantly (P<0.05) reduced in +Zn compared to other groups. Intestinal glutathione (GSH) levels were found to be high in +Zn but lowered significantly in +Fe+Zn. Lowest concentration of GSH was found in +Fe. Serum a-tocopherol concentration was highest in +Zn group.
2. Zinc repletion alone or together with iron resulted in induction of intestinal metallothionein both in zinc and combined deficiency.
3. Serum ceruloplasmin activity was higher in +Fe+Zn but similar in both +Fe and +Zn groups.
4. Immuno-histochemical localization of ferritin, transferrin, metallothionein and micro autoradiographic presence of iron and zinc at the site absorption favor lowered iron-induced stress.
5. Separate experiment with control intestinal mucosal and luminal contents showed that zinc per se reduced iron induced hydroxyl radical production, indicating possible mechanism (Figure - 7).

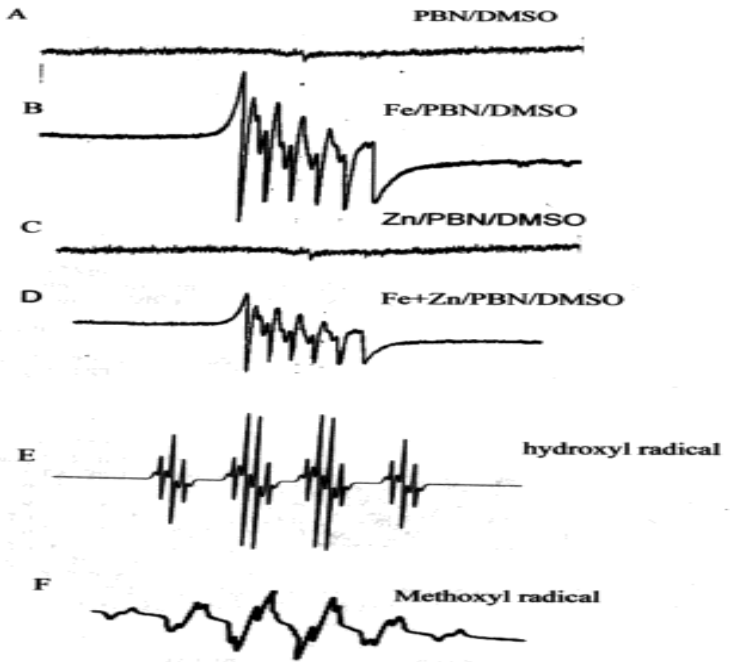


Figure.7 : EPR spectra showing that zinc per se reduces iron induced hydroxyl radical production. Panel (A) Vehicle control (B) with Fe (C) with Zn (D) with Fe+Zn (E & F) Computer simulated pattern of the spectra (E) for hydroxyl radical and (F) for methoxyl radical.

CONCLUSION

These findings suggest that zinc deficiency per se compromise deficiency of iron and combined repletion not only improved iron and zinc status but also contributed positively in reducing the oxidative stress. This appears to be due to interaction of both iron and zinc at the site of absorption and to an increased synthesis of metallothionein (increased scavenging ability of free radicals). Therefore, concurrent supplementation of iron and zinc seems to be more beneficial in correcting deficiencies of iron / or zinc.Nutrition and Infection

2. ESTABLISHMENT OF A NATIONAL FACILITY FOR DRIED BLOOD SPOT (DBS) TECHNOLOGY FOR VITAMIN A ESTIMATION

Investigators : B.Sivakumar and K.Madhavan Nair

Duration : 2 years

Date of completion : December 2003

BACKGROUND

For assessing vitamin A deficiency, WHO and UNICEF have recommended the use of serum retinol levels. In field conditions obtaining venous sample becomes very difficult owing to inherent operational problems. To overcome some of these problems, many researchers are working for alternate methods. Craft and Co-workers from Crafts Technology, USA has evolved a method for collection of blood sample on to a special type of filter paper using finger prick. These filter papers can be dried and easily transported under cold conditions to a laboratory. Thus, large number of samples can be collected and analysed by this DBS method. Hence, DBS technology is an ideal method for collecting blood samples for assessing vitamin A status in a given population and to evaluate the impact of vitamin A supplementation programmes aimed at reducing vitamin A deficiency. The project was taken up with the following objectives:

1. To set up a laboratory for estimation of serum vitamin A using dry blood spot by HPLC methodology.
2. To provide technical services for analysis of vitamin A atleast 2000 samples/year on charge basis.

METHODOLOGY

The facility has been established with the financial support from Micronutrient Initiative. The facility consists of 2 HPLC system (one gifted from MOST) with auto samplers, a sonicator water bath, spectrophotometer and cold centrifuge. Both the HPLCs were validated and analysis of vitamin A from serum and fortified sugar were performed. Standardization of the method for analyzing retinol from DBS is completed. Briefly, the method involved extracting vitamin A by sonication from a known area of the blood spot (0.635 cm disk) punched from the DBS. Tocol was used as an internal standard to check recovery. Blood sample with known retinol concentration and spotted on to filter paper and stored along with other samples was used to know the loss of retinol during storage.

RESULT

Analytical performance characterstics of the method is provided in Table 3. A SOP for the method has been prepared. The recovery of the retinol from the spot was above 90%.

Analytical Performance Characteristics	
Detection limit	3.0 µg/dL
Quantation limit	4.3 µg/dL
Range	4.3 – 70 µg/dL
Analyate recovery	97
Intra assay variability	<6%
Inter assay variability	<6%
Validity (at 20 µg/dL) **Sensitivity** **Specificity**	73-93% 90-100%
Compared to serum retinal (r^2)	0.95
cost	Rs. 800

CONCLUSION

A facility to estimate serum vitamin 'A' by DBS technology has been established. The cost of analysis for each sample is estimated to be around Rs. 800. The facility is expected to generate its own funds by undertaking analysis of DBS for vitamin A under various projects.

3. FORTIFICATION OF SUGAR WITH MICRONUTRIENTS

Investigators : S. Ranganathan

Duration : 4 years

Date of completion :

BACKGROUND

Sugar has been identified as a vehicle for micronutrient fortification. Vitamin A fortified sugar is widely accepted in countries like Costa Rica, Guatemala, Honduras, and Panama. The National data on sugar consumption shows that the intake is reasonably good in the country (15–30g). Further, the consumption of soft drinks and confectionery is increasing in the country. Due to the dry crystalline nature of Sugar, it has a great advantage over other vehicles for micronutrient fortification and its interference with the absorption of nutrients like iron is negligible. Thus, developing an indigenous technology for sugar fortification will be very useful for the country so that micronutrients can be delivered to selected populations such as children and adolescents through foods prepared from fortified sugar.

OBJECTIVES

To develop an indigenous technology for the fortification of sugar with micronutrients.

Sugar and fortificants

Refined crystal sugar from the local market was used for fortification. Potassium iodate (BDH, IP grade) was used as iodine source. Ferrous sulphate hepatahydrate (Allied Chemicals & Scientific Co., Hyderabad, IP grade), ferrous glycine sulphate (Medcell Laboratories, Chennai, IP grade) and dried ferrous sulphate, & ferrous fumerate (Nicolas Primal India Ltd., Thane, IP grade) were used as iron sources.

TECHNOLOGY

1. Iodine fortified sugar:

Spray mixing was followed. Three kg sugar was taken in a plastic basin (10 kg) and sprayed with a solution of KIO_3 (75 mg) dissolved in 3 ml water using a glass micropipette and mixed well for uniform blending of Iodine (15 ppm). In the case of 30 ppm iodine, 3 kg sugar was spray mixed in a similar fashion with 3 ml solution of KIO_3 (150 mg) in water.

2. Iron fortified sugar:

Dry mixing was followed. Fortification was done at at 500 ppm & 1000 ppm iron levels. Three kg of iron-fortified sugar was prepared with each iron source at each iron level. Required amount of iron chemicals for 3 kg sugar are: 15 g Ferrous sulphate hepatahydrate, 15 g ferrous glycine sulphate, 9.75 g dried ferrous sulphate, and 9.75 g ferrous fumerate for 1000 ppm iron and half of these quantities for 500 ppm iron. First 30 g sugar was taken in a plastic basin (10 kg) and mixed well with the iron chemical needed for 3 kg sugar and then the remaining sugar was added ((2770 g) by quantitative addition and mixed well for uniform blending of iron (500 ppm iron or 1000 ppm iron).

3. Iron & Iodine fortified sugar:

Dry mixing for iron fortification and spray mixing for iodine fortification were followed. First, 3 kg of iron-fortified sugar was prepared with each iron source at a level of 1000 ppm iron by dry mixing method in a plastic basin as described for iron fortified sugar. Then it was spray mixed with 3 ml solution of KIO_3 (150 mg) dissolved in water in the same plastic basin as described for iodine fortified sugar.

Iodine & iron estimations

Iodine in the fortified sugar was estimated by the titration method of ICCIDD and iron was estimated by the Wong's method.

Stability study

All the fortified sugars were stored in 1 kg double capped plastic containers (screw-capped with inner cap) protected from light and heat at ambient temperature. At 3 monthly intervals, the color, iodine content and iron content of fortified sugars were monitored.

Results

Iodine fortified sugar:

Iodine was stable at the initial level (15 ppm or 30 ppm) at 12 months (Table –4). No color difference was observed by visual comparison between unfortified and iodine fortified sugars.

Iron fortified sugar:

The initial iron content (500 ppm or 1000 ppm) was retained at 12 months (Table - 5). The pH of 5% aqueous solution depended on the source of iron. Though there was no visible color differences initially between unfortified and iron fortified sugars, slight visible unacceptable discoloration was observed after six months in sugars fortified with ferrous sulphate hepatahydrate, dried ferrous sulphate, and ferrous fumerate. However, ferrous glycine sulphate fortified sugar did not show any discoloration at 12 months.

Iron & Iodine fortified sugar:

The initial iodine level (30 ppm) was retained at 12 months in double fortified sugar prepared from ferrous glycine sulphate. But, there was 50% - 70% iodine loss at 12 months in double fortified sugars prepared from other iron sources (Table – 6). The initial iron content (1000 ppm) was retained at 12 months (Table – 7). After six months slight visible unacceptable discoloration was observed in double fortified sugars fortified with ferrous sulphate hepatahydrate, dried ferrous sulphate, and ferrous fumerate. However, there was no discoloration of double fortified sugar prepared from ferrous glycine sulphate.

Table 4. Stability of iodine in iodine fortified sugar

pH*	Iodine content (ppm)**				
	Initial	3 months	6 months	9 months	12 months
6.0	15.5 ± 0.4	15.7 ± 0.7	15.4 ± 0.6	15.5 ± 0.3	15.4 ± 0.3
6.1	30.1 ± 0.4	31.4 ± 0.6	30.2 ± 0.5	30.1 ± 0.4	29.8 ± 0.6

* pH of 5% aqueous solution; ** Mean ± SD, n = 6.

Table 5. Stability of iron in iron fortified sugar

pH*	Iron source	Iron content (ppm)**				
		Initial	3 months	6 months	9 months	12 months
3.9	FGS	498 ± 18	501 ± 18	504 ± 14	502 ± 11	497 ± 6
5.3	FSHH	506 ± 11	499 ± 13	503 ± 17	500 ± 13	505 ± 7
5.7	Dried FS	505 ± 14	507 ± 8	496 ± 11	505 ± 13	499 ± 11
6.1	FF	495 ± 14	498 ± 12	490 ± 7	505 ± 6	494 ± 6
3.8	FGS	999 ± 6	978 ± 18	974 ± 39	1032 ± 17	989 ± 6
5.2	FSHH	995 ± 21	1009 ± 16	999 ± 8	997 ± 20	1017 ± 18
5.6	Dried FS	1005 ± 14	999 ± 11	987 ± 24	997 ± 24	997 ± 18
5.9	FF	1001 ± 18	1002 ± 11	1003 ± 25	983 ± 18	998 ± 25

* pH of 5% aqueous solution; ** Mean ± SD, n = 6.
FGS: Ferrous Glycine sulphate; FSHH: Ferrous sulphate hepatahydrate;
Dried FS: Dried ferrous sulphate; FF: Ferrous fumerate.

Table 6. Stability of iodine in double fortified sugar

pH*	Iron source	Iodine content (ppm)**				
		Initial	3 months	6 months	9 months	12 months
3.8	**FGS**	29.4 ± 2.5	30.3 ± 1.9	30.8 ± 2.5	30.0 ± 2.1	30.0 ± 2.3
5.2	**FSHH**	32.2 ± 3.0	20.9 ± 1.0	16.2 ± 1.2	12.1 ± 0.9	10.7 ± 0.7
5.6	**Dried FS**	26.1 ± 2.1	19.5 ± 2.0	16.8 ± 1.3	12.3 ± 0.7	10.5 ± 0.8
5.9	**FF**	30.5 ± 2.5	20.7 ± 1.0	15.7 ± 0.3	10.5 ± 0.7	9.3 ± 0.7

* pH of 5% aqueous solution; ** Mean ± SD, n = 6.
FGS: Ferrous Glycine sulphate; FSHH: Ferrous sulphate hepatahydrate;
Dried FS: Dried ferrous sulphate; FF: Ferrous fumerate.

Table 7. Stability of iron in double fortified sugar

pH*	Iron source	Iron content (ppm)**				
		Initial	3 months	6 months	9 months	12 months
3.8	**FGS**	1010 ± 14	995 ± 21	1000 ± 14	1007 ± 11	1015 ± 7
5.2	**FSHH**	1005 ± 7	1010 ± 14	993 ± 11	1012 ± 10	1025 ± 28
5.6	**Dried FS**	978 ± 25	985 ± 21	975 ± 7	970 ± 7	970 ± 14
5.9	**FF**	1012 ± 39	1010 ± 14	1000 ± 14	1010 ± 14	1005 ± 14

* pH of 5% aqueous solution; ** Mean ± SD, n = 6.
FGS: Ferrous Glycine sulphate; FSHH: Ferrous sulphate hepatahydrate;
Dried FS: Dried ferrous sulphate; FF: Ferrous fumerate.

CONCLUSION

1. Iodine fortified sugar, prepared by spray mixing, showed good iodine stability at 12 months both at 15 ppm and 30 ppm iodine levels. No discoloration was observed.
2. Ferrous glycine sulphate was found to be a better source for iron fortified sugar as there was no discoloration.
3. Double fortified sugar prepared from ferrous glycine sulphate showed good stability of iodine.
4. Iron stability remained unaltered at 12 months both in iron fortified and double fortified sugars.
5. Acceptability study of fortified sugars in food preparations is completed and the data will be presented.

4. EFFECT OF DIETARY ALTERATION OF n-6 AND n-3 POLYUNSATURATED FATTY ACIDS ON INSULIN RESISTANCE, STRUCTURE AND FUNCTION OF ADIPOCYTES AND SKELETAL MUSCLE

Investigators : Ghafoorunissa & Ahamed Ibrahim

Duration : 3 years

Date of completion :

Insulin resistance is a common metabolic abnormality that is implicated in the development of several diet-related chronic diseases including type 2 diabetes, hypertension, obesity and coronary heart disease. Skeletal muscle and adipose tissue are the major target tissues for insulin action. Binding of insulin to the target tissue results in autophosphorylation of the receptor and activation of tyrosine kinase which in turn phosphorylates several intracellular substrates resulting in transport of glucose. Dietary fatty acids affect depot lipids (triglycerides) and membrane lipids. The composition of membrane lipids affects a range of biochemical processes either directly (fluidity, receptor binding etc) or via production of eicosanoids. Recent studies suggest that skeletal muscle and adipose tissue phospholipid fatty acid compositions affect insulin sensitivity. While saturated fatty acids (SFA) may have detrimental effects, the long chain polyunsaturated fatty acids (LCPUFA) may potentiate insulin action. The n-6 and n-3 PUFA have distinct biological effects and therefore both absolute levels and optimal ratio (n-6/n-3) are essential for blood vascular homeostasis, optimal immune functions and lipoprotein metabolism.

AIMS AND OBJECTIVES

To investigate the effects of increasing dietary LCn-3PUFA and therefore alterations of n-6/n-3 ratio (linoleic acid (18:2n-6)/LCn-3 PUFA) on adipose tissue and skeletal muscle lipid composition, fluidity and insulin action in sucrose induced insulin resistant rat model.

METHODOLOGY

80 WNIN weanling rats were divided into five groups (16 animals in each group) and fed casein based diet containing 10% fat. Insulin resistance was induced by replacing starch with sucrose (55% total diet). Vegetable and fish oil blends used as dietary fat source furnished: saturated fatty acids ~6en%, monounsaturated fatty acids ~ 9en%, PUFA (18:2 n-6 + LC n-3 PUFA) ~ 6en% and P/S ratio ~ 1. The ratio of 18:2n-6/ LC n-3 PUFA in various groups were as follows:

Group I	:	Starch	= 200
Group II	:	Sucrose	= 200
Group III	:	Sucrose	= 50
Group VI	:	Sucrose	= 10
Group V	:	Sucrose	= 5

After three months feeding, blood was collected after overnight fasting and animals were sacrificed. Epididymal and retroperitoneal fat pads and diaphragm were removed. Adipocytes were isolated from retroperitoneal and epididymal fat pads. Plasma membrane was prepared from adipocytes by density gradient centrifugation. The following parameters were studied:

1. Plasma glucose and insulin after oral glucose tolerance test (OGTT)
2. Plasma triglycerides, cholesterol and HDL cholesterol.
3. Adipocyte number and size.

4. Adipocyte plasma membrane lipid composition (cholesterol, phospholipids), fluidity and fatty acid composition of phospholipids.
5. Skeletal muscle triglycerides and phospholipid fatty acid composition
6. Adipocyte lipolytic activity and antilipolytic effect of insulin
7. Adipocyte and skeletal muscle glucose transport.
8. Adipocyte insulin binding

The results were analyzed by one-way ANOVA

1. Compared to starch, sucrose feeding significantly increased body weight gain, epididymal and reteroperitonial fat weights. However decreasing 18:2n-6/LC n-3 PUFA ratio did not have any effect.

2. Plasma glucose and area under the curve for glucose after OGTT were similar in all groups.

3. Sucrose feeding significantly increased basal levels of plasma insulin and area under the curve for insulin after OGTT. Decreasing 18:2n-6/LCn-3 PUFA to 10 and 5 decreased insulin levels and area under the curve for insulin to values similar to starch fed controls.

4. Compared to starch, sucrose feeding significantly increased plasma triglycerides. Decreasing 18:2 n-6/LCn-3 PUFA ratio to 50, 10 or 5 decreased plasma triglycerides to similar extent.

5. Plasma total cholesterol and HDL cholesterol were not altered by sucrose feeding. Decreasing 18:2 n-6/LCn-3 PUFA ratio to 10 or 5 significantly decreased plasma total cholesterol to similar extent. However HDL cholesterol decreased only at dietary ratio of 5.

6. Adipocyte plasma membrane cholesterol, phospholipids and fluidity were similar in all groups.

7. The data on adipocyte plasma membrane fatty acid composition showed dose dependant increase in docosapentaenoic (22:5n-3) and docosahexaenoic (22:6n-3) acids. With dietary 18:2n-6/LCn-3 PUFA ratio of 10 and 5 the levels of arachidonic acid (20:4n-6) decreased.

8. Basal and norepinephrine induced lipolysis were higher in sucrose fed group compared to starch fed group. Decreasing 18:2n-6/LCn-3PUFA ratio to 10 or 5 decreased both basal and norepinephrine induced lipolysis to the same extent.

9. Sucrose feeding resulted in decrease in adipocyte sensitivity to the antilipolytic effect of insulin. Decreasing 18:2n-6/LCn-3 PUFA ratio to 10 or 5 increased the adipocyte sensitivity to the antilipolytic effect of insulin.

10. Compared to starch, sucrose feeding decreased insulin stimulated glucose transport. Decreasing 18:2n-6/LCn-3 PUFA ratio to 10 or 5 increased adipocyte insulin stimulated glucose transport to the same extent.

11. Neither sucrose feeding nor decreasing 18:2n-6/LCn-3 PUFA ratio affected skeletal muscle glucose transport.

Analysis of adipocyte size and number, insulin binding and skeletal muscle phospholipid fatty acid composition are in progress. The above findings suggest that 0.5en% LCn-3 PUFA (18:2n-6/ LCn-3PUFA = 10) may be needed to prevent diet-induced insulin resistance.

Earlier studies showed that 0.7en% 18:3n-3 (combination of cooking oils) or 0.1en% LCn-3 PUFA (fish oil supplements) improved n-3 PUFA nutritional status and decreased platelet aggregation in Indian subjects (Ghafoorunissa et al, Lipids, 2002). The studies on effects of dietary n-3 PUFA on diet-induced insulin resistance in rats showed that 2en% a-linolenic acid (18:3n-3) (Annual Report 2001-02 & 2002-03) or 0.5en% LCn-3 PUFA (present study) may be needed for prevention of insulin resistance. It therefore appears that for prevention of insulin resistance and its sequaele leading to chronic diseases, the present intake of 18:3n-3 in Indian subjects (<0.5en%) (Ghafoorunissa, IJMR 1998) should be increased to 1–2en%. When fish oil supplements are used these should provide 0.1-0.5en% LCn-3 PUFA. Studies have been initiated on the preventive and therapeutic benefits of 1-2 en% 18:3n-3 or 0.1-0.5en% LCn-3PUFA in diabetes or in subjects with impaired glucose tolerance.

5. RESISTIN: A MOLECULAR LINK BETWEEN TYPE2 DIABETES AND OBESITY

Investigators : Nasreen Z. Ehtesham, Sudip Ghosh and Sangita Mukhopadhyaya (CDFD), B.Aruna, Anil K Singh (PhD students)

Duration :

Date of completion :

BACKGROUND

Type 2 diabetes is a multifactorial, polygenic disorder characterized by chronic hyperglycemia arising from insulin resistance, where target tissues fail to respond to normal level of insulin. Epidemiological observations underscore a strong correlation of obesity with type 2 diabetes. Recently, resisitn, a cysteine rich secretory protein, which is down regulated by anti-diabetic drugs like thiazolidinediones (TZDs), has been implicated as the link between NIDDM and obesity in mouse. Although the human and the mouse protein share significant sequence homology (59%), the role of human resistin in the etiology of type 2 diabetes and obesity have been controversial. Several human studies have demonstrated lack of correlation between the circulating levels of resistin and obesity, whereas, in others increased levels of resistin was correlated with visceral obesity. Although, resistin is a small polypeptide of about 12 kDa, it has 11 cysteine residues at highly conserved positions. The physiological role of resistin can be better understood with knowledge of its molecular features.

RESULTS

Earlier we cloned, expressed and purified human resistin to homogeneity from inclusion bodies. The protein was found to be unusually stable because of intermolecular disulfide linkages. Human resistin showed a tendency to aggregate at higher concentration and undergoes dynamic structural changes in a concentration and time dependent manner. At lower concentration it possessed a predominantly a-helical structure. However, as the concentration of the protein increased, a significant shift towards the b-conformation was observed. The concentration dependent change in structure is a reminiscent of many proteins that exert their pathophysiological property through a conformational switch e.g. prion proteins, amyloids etc. However, resistin differs from these proteins in terms of its ability to reverse the aggregation when diluted. This may have important implications on how resistin exerts its pathophysioloical effect (Aruna B. et. al., 2003, *Biochemistry*, 42: 10554-10559).

To understand the biophysical nature of resistin we further determined its quaternary structure from freshly refolded proteins. We purified resistin using a qasi-static like process as well as on-column refolding method, and resistin was found to exist predominantly as dimer (Fig. 8) suggesting inherent dimeric nature of resistin. Further analyses revealed that dimerization of resistin involves both covalent and non-covalent interaction. The presence of monomeric form in SDS-PAGE under non-reducing condition confirm the involvement of non-covalent linkages in dimerization. The dimeric form was much higher when electrophoresed under non-reducing condition (Fig. 9A) as compared to reducing condition, indicating that dimerization is partly by covalent linkage involving disulfide bonds. This result was further confirmed by Western Blot analysis using anti-resistin antibodies raised in rabbit (Fig. 9B). Thus, our results have shown that human resistin dimerizes spontaneously through covalent and non-covalent interactions (Raghu et al., 2004, *BBRC,* 313 :642–646).

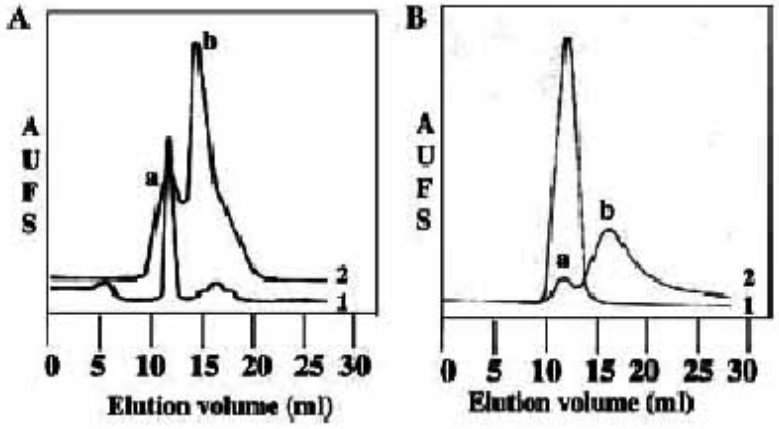


Figure 8. Gel filtration analysis of recombinant resistin reveals its dimeric nature: Purified recombinant resistin was loaded on Sephadex G-50 gelfiltration column. Standard molecular markers-carbonic anhydrase (29 kDa) and cytochrome C (12.7kDa)-are represented by peaks a and b of curve 2 respectively. Curve 1 represents gelfiltration analysis of (A) quasi-static refolded protein (B) on-column refolded protein

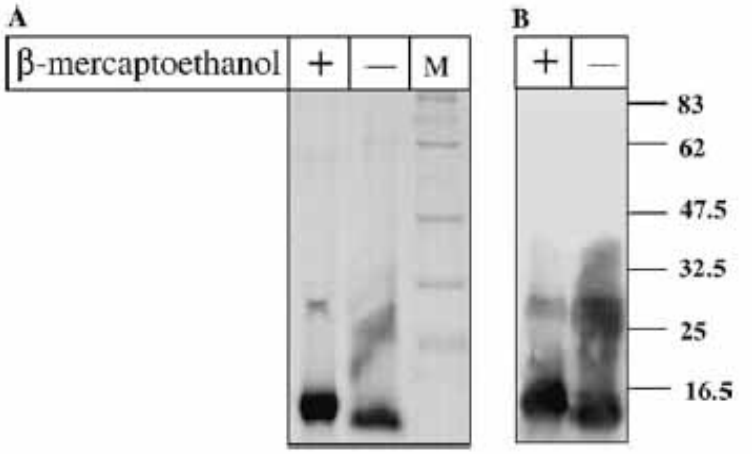


Figure 9: Dimeric resistin has both covalent and non-covalent interactions: Purified recombinant resistin was incubated with sample buffer with (+) or without (-) 20% ß-mercaptoethanol and separated on 10% SDS-PAGE using Tris-tricine buffer. Coomassie stained gel is represented in A and immunoblot is shown in B.

6. FOETAL METABOLIC PROGRAMMING FOR INSULIN RESISTANCE SYNDROME: ROLE OF MICRONUTRIENTS IN MATERNAL DIET

Investigators : L.Venu, T.Prasanna Krishna and M.Raghunath

Duration : 2 years

Date of completion : August 2003

Insulin Resistance (IR), the condition in which the target cells of insulin become increasingly insensitive to insulin stimulated glucose uptake, is central to IR Syndrome. IR Syndrome or the metabolic syndrome is a significant contributor to the increasing incidence in India, of coronary artery disease, hypertension and type 2 diabetes.

Under nutrition of the foetus during gestation and neonatal / perinatal periods, which is common in developing countries like India, has been proposed to be important in the development of IR Syndrome in adult life. However, the precise identity of the maternal nutritional factors (specially the micronutrients, whose deficiencies are widespread during pregnancy / lactation), which predispose the offspring to Syndrome X in later life, is not clear yet. From the evidence available we hypothesize that maternal dietary micronutrient deficiency *per se* predisposes the offspring to IRS in later life. Therefore, this study has been undertaken to validate/negate the above hypothesis with the following objective.

OBJECTIVE

Assess the role of maternal dietary micronutrient (minerals and vitamins) restriction *per se* on the development of insulin resistance syndrome in the later life of the offspring.

A. Experiment # 1: Effect of maternal dietary mineral restriction

The aim of this experiment was to assess the effect of 50% mineral restriction / rehabilitation in mother's diet on the development of Insulin Resistance syndrome in offspring.

Female, weanling WNIN rats were fed for 12 weeks (n=14), a 20% protein diet containing mineral mixture at 50% level of that of control diet, and the serum levels of important minerals like Fe, Zn, Cu, Mg and Ca were determined at the end of the feeding regimen. Then they were mated with control WNIN males and the pregnant dams continued on mineral restricted diet throughout their pregnancy. At parturition, four rats from the restricted group were shifted to control diet (MSP), while the remaining mothers continued on restricted diet till weaning. At this point, half the numbers of the offspring were weaned on to control diet (MSW), while the other half were weaned on to restricted diet (MR). Appropriate mineral control animals (MC) (n=6) were maintained throughout the experiment. The effect of maternal dietary mineral restriction / rehabilitation was evaluated on the following IR related parameters on post-natal day 40,70, 100 and 180 of the offspring.

Parameters studied:

I. In mothers
   - i) Plasma levels of Fe, Zn, Cu, Mg and Ca, cholesterol and triglycerides.
   - ii) Fasting blood glucose and insulin at the time of mating.
   - iii) Reproductive performance
   - iv) Oral glucose tolerance at the weaning of the pups.

II. In pups on post-natal day 40, 70, 100 and 180.
   - i) Food intake, Body weight; Haemoglobin and BMI (at 100 & 180 days only).
   - ii) Oral glucose tolerance and Plasma insulin levels at 0, 1, 2 hrs.

iii) Insulin resistance indices: HOMA Index and ratio of Glucose AUC / Insulin AUC during OGTT.
iv) Hemoglobin, Total cholesterol, HDL cholesterol & triglycerides.
v) Body composition measurement by Total Body Electrical Conductivity TOBEC) (at 100 & 180 days only).
vi) Oxidative Stress (MDA, Protein Carbonyls and Reduced Glutathione) in liver.
vii) Antioxidant Enzymes (Catalase, SOD and GPx) in liver.

RESULTS

The results are summarized below:

i) There were no significant differences among mothers of different groups in their diet intake or weight gain, while significant decrease was observed in hemoglobin and serum levels of iron, zinc, magnesium and calcium. The oral glucose tolerance and serum lipid levels were normal and comparable among mothers of different groups.

ii) Weight gain during pregnancy and litter size was comparable among different groups. However 14 % (2/14) of mothers only in MR group had abortions.

iii) The mean birth weight of pups was significantly lower in MR group than controls ($p<0.01$)(5.52 ± 0.11g vs. 6.08 ± 0.14g). They continued to weigh less till postnatal day 180, whether continued on MR or shifted to control diet from parturition or weaning. Although there were no significant differences in Body Mass Index (BMI) among the offspring of different groups measured on postnatal day 100, the offspring of MR, MSP and MSW had lower BMI compared to control at postnatal day 180 ($p<0.05$).

iv) Maternal mineral restriction in general had no significant effect on fasting glucose and insulin levels till postnatal day 70. However on postnatal day 100 and 180, MR, MSP and MSW offspring had significantly lower values for fasting glucose but not insulin, compared to controls. Although the HOMA IR values computed were not statistically significant among the groups, were lower in MR, MSP and MSW offspring compared to controls.

v) In keeping with the above observations, there were no differences among the offsprings of different groups in their OGT till postnatal day 100. However, on postnatal day 180, the MSP offspring had the highest AUC of glucose while the MC, MR and MSW pups were comparable. Interestingly MR, MSP and MSW offspring had lower AUC of insulin compared to controls (of which only MSP and MSW were significant) and this probably indicates that maternal mineral restriction decreased the capacity of the offspring to respond to a challenge of oral glucose and rehabilitation from parturition or weaning may not mitigate the defect.

vi) Body fat percent was significantly higher ($p<0.01$) in MR offspring compared to controls (Figure 10 Panel A). Other markers of adipogenesis like lean body mass and fat free mass (Figure 10 Panels B & C) were significantly lower ($p<0.001$) in the MR offspring compared to controls. However, rehabilitation of MR dams from parturition (MSP) or weaning MR pups on to control diet (MSW) seemed to have no significant effect on any of these parameters at both the time points tested.

vii) In keeping with high body fat %, plasma triacylglycerol concentrations were significantly higher in the mineral restricted offspring (MR) than controls (Figure 10 Panel D)

(p<0.05) at all the four time points tested. Interestingly, mineral rehabilitation from parturition (MSP) or weaning (MSW) appeared to correct the changes almost completely. Plasma total cholesterol and HDL cholesterol, in general were not significantly different among the groups at any of the time points tested (postnatal day 100&180).

viii) Chronic maternal mineral restriction resulted in a significant (p<0.05) decrease in reduced glutathione (GSH) levels in the MR offspring and an increase in protein carbonyls albeit not statistically significant. Interestingly, rehabilitation from parturition or weaning could not prevent either the decrease in glutathione or the increase in the concentrations of protein carbonyls. Lipid peroxides (MDA) and activities of antioxidant enzymes Catalase, SOD and GPx were similar among all the groups.

In the present study, although maternal mineral restriction had no effect on glucose metabolism in the offspring, significant increases were observed in plasma triacylglycerols and body fat percent. The high body fat % along with low body weight in the MR offspring observed here appear similar to those reported in the "thin fat babies" seen in developing countries like India, an abnormal condition attributed to maternal malnutrition. Though it is not immediately clear how maternal mineral restriction altered lipid metabolism, it appears that it may not be due to the increased oxidative stress in these animals. In view of the earlier literature that changes in adipogenesis / fat metabolism are the earliest events in the manifestation of IR, our observations appear to support the hypothesis that maternal dietary mineral nutrition *per se* may be important in predisposing the offspring to insulin resistance syndrome in later life.

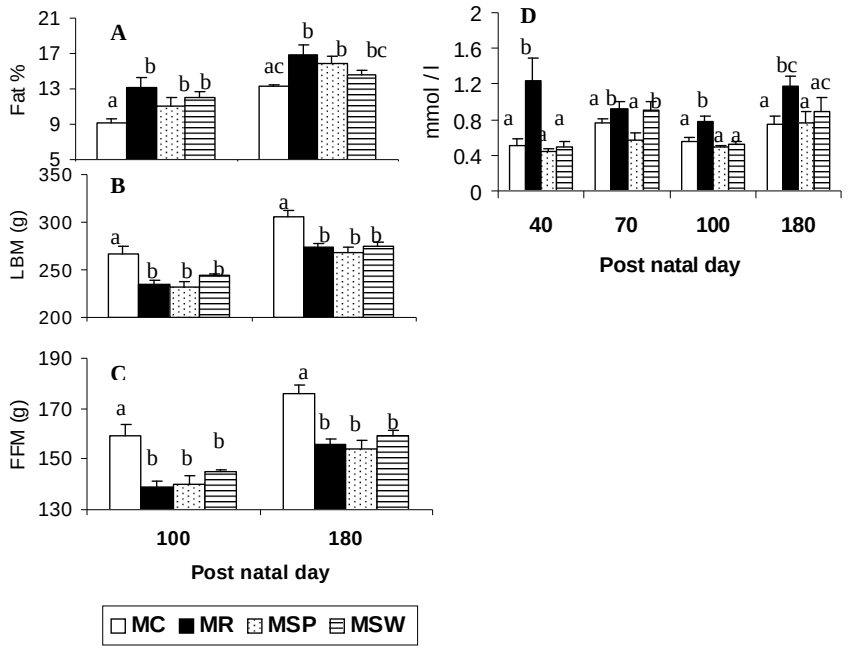


Figure 10. Body Fat % **(panel A),**
Lean Body Mass **(panel B)**
Fat free mass of the offspring on postnatal day 100 and 180 as determined by TOBEC **(panel C)**

Plasma triacylglycerols in the offspring of different groups on postnatal days 40, 70, 100 and 180 **(Panel D)**
Values are mean ± SE (n=6).
Means at an age without a common letter are significantly different at $p < 0.05$ by ANOVA / Post Hoc LSD

B. Experiment # 2: Effect of maternal dietary vitamin restriction.

This experiment was conducted to assess the effect of maternal dietary vitamin restriction to 50 % of controls in predisposing the offspring to Insulin resistance.

The number of dietary regimens (groups), number of animals per group, duration of feeding of different diets to mothers / offspring and the different parameters monitored were similar to those of the earlier experiment, excepting that experimental animals were fed for 12 weeks 20% protein diet containing vitamin mixture at 50% level of that of control diets. The vitamin status of the mothers was monitored by determining their plasma levels of vitamin A, vitamin E and Folic acid. The parameters monitored in the offspring and the post-natal days on which they were monitored were same as in the previous experiment.

RESULTS

The results of this experiment are summarized below:

i) There were no significant differences among dams of different groups in their diet intake or weight gain. Vitamin restricted (VR) animals had significantly lower hemoglobin and plasma vitamin E levels. However the OGT and plasma lipid levels were normal and comparable among the mothers of different groups.

ii) Weight gain during pregnancy and litter size was normal and comparable in control and VR dams.

iii) Despite similar birth weights, body weights of VR & VSP pups were significantly lower at weaning compared to controls ($p<0.05$). While VR offspring continued to weigh less till postnatal day 180, those shifted to control diet from parturition and weaning caught up with controls in their body weight. However, there were no differences among the offspring of different groups in their body mass index (BMI) measured on postnatal day 100 and 180.

iv) There were no significant differences among the offspring of different groups of the offspring in the fasting glucose levels till postnatal day 100, but for some transient changes seen in VR pups on postnatal day 40. However, on postnatal day 180, the VSW group had significantly higher fasting glucose levels ($P<0.01$) compared to control. However, there were no significant differences in fasting insulin levels among the four groups at any of the time points tested

v) The area under the curve for glucose and insulin during an OGTT, in general were not significantly different among the four groups till PN day 100, but for some transient changes seen in VR on PN day 40 (AUC glucose) and 70 (AUC insulin). Although not significant statistically, on postnatal day 180, VSP and VSW offspring had higher AUC of glucose whereas VR and VSW offspring had lower AUC of insulin, compared to controls.

vi) In general, VR pups were not significantly different from controls in both the above indices at any of the four time points tested, nor did rehabilitation from parturition (VSP) or weaning (VSW) had any impact on these indices. However, at 180 days of life the

ratio of AUC of glucose to that of insulin was higher in VR and VSW (P<0.01) than that of VC and VSP. These results probably indicate that maternal vitamin restriction decreased the capacity of offspring to respond to a challenge of oral glucose vis a vis insulin secretion and rehabilitation from weaning may not mitigate the defect.

vii) Compared to controls, body fat percent was significantly higher (P<0.01) in VR offspring (Figure 11 Panel A) and other markers of adipogenesis like lean body mass and fat free mass (Figure 11 panels B&C) were significantly lower (P<0.05) on postnatal day 100 & 180. In keeping with high body fat%, plasma triacylglycerols levels were significantly higher in VR offspring than controls (P<0.01) (Figure 11 Panel A) from postnatal day 70 onwards. Rehabilitation of VR dams from parturition (VSP) but not weaning the VR pups on to control diet (VSW) corrected the changes almost completely by postnatal day 180. Plasma total cholesterol and HDL cholesterol in the offspring, in general, were not significantly different among the groups at any of the time points tested.

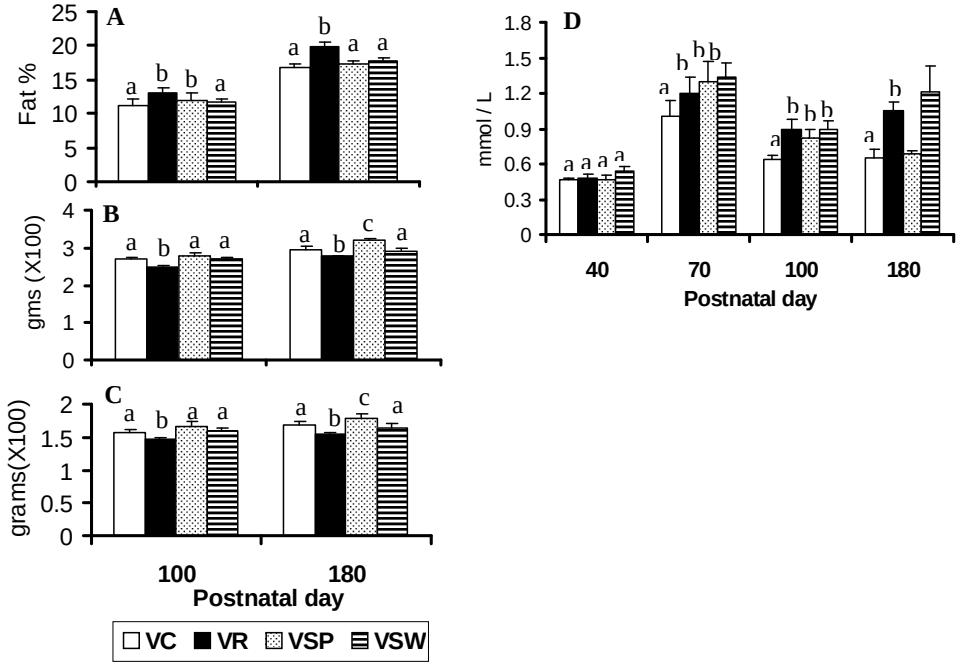


Figure 11. Body Fat % **(panel A)**
Lean Body Mass **(panel B)**
Fat free mass of the offspring on postnatal day 100 and 180 as determined by TOBEC **(panel C)**
Plasma triacylglycerols in the offspring of different groups on postnatal days 40, 70, 100 and 180 **(Panel D)**

Values are mean ± SE (n=6).
Means at an age without a common letter are significantly different at p < 0.05 by ANOVA / Post Hoc LSD

i) Chronic maternal vitamin restriction resulted in a significant increase in levels of MDA (p<0.01) and protein carbonyls albeit not significant (Table 8). Despite that liver SOD and GPx activities were significantly higher in VR offspring than controls (P<0.001) (Table 8), the increased oxidative stress in them appears to be due to a significant decrease in reduced glutathione (GSH) levels (p<0.05).

Table 8. Indices of oxidative stress, antioxidants and antioxidant enzymes in liver homogenate of offspring of different groups on postnatal day 180

Parameter	VC	VR	VSP	VSW
Malondialdehyde *(nmol/mg protein)*	0.106±0.003[a]	0.159±0.02[b]	0.13±0.01[ab]	0.102±0.007[a]
Protein Carbonyls *(nmol/mg protein)*	3.95±0.43[a]	4.80±0.43[a]	5.31±1.10[a]	5.00±0.40[a]
Reduced Glutathione *(μmol/mg protein)*	2.85±0.24[a]	1.83±0.26[b]	2.94±0.24[a]	3.03±0.41[a]
Antioxidant Enzymes (Units / mg protein)				
Catalase	4.66 ± 0.51[a]	4.69 ± 0.51[a]	4.37 ± 0.53[a]	6.28 ± 1.10[a]
Superoxide Dismutase	14.88 ± 0.41[a]	17.68 ± 0.27[b]	14.32 ± 0.34[a]	17.60 ± 0.57[b]
Glutathione Peroxidase	0.137±0.018[a]	0.210±0.019[b]	0.185±0.011[b]	0.120±0.013[a]

Values are mean ± SE (n=6).
Means at an age without a common letter are significantly different at $p < 0.05$ by ANOVA / Post Hoc LSD.

Interestingly, rehabilitation from parturition or weaning could partially prevent the changes in MDA and GSH levels but not that of protein carbonoyls. The increased SOD could be corrected by rehabilitation from parturition but not weaning, whereas both appeared to correct the increase in GPx. Abundant literature indicates that altered adipogenesis / lipid metabolism is the earliest change seen, much before tissue insulin resistance manifests. Indeed, insulin resistance is hypothesized to originate in impaired adipogenesis / lipid metabolism. Although the VR offspring had neither impaired glucose tolerance nor insulin resistance till post natal day 180, the finding that they had significantly high body fat content along with altered lipid metabolism appears to suggest that these offspring were probably predisposed to insulin resistance in their later life.

The results of the two experiments suggest a role for maternal micronutrient status during pregnancy and lactation on the predisposal of offspring to insulin resistance in later life and appear to support the hypothesis that maternal dietary micronutrient restriction *per se* may predispose the offspring to IRS in later life.

7. EFFECT OF DIET-RESTRICTED, MICRONUTRIENT-SUFFICIENT DIET ON LONGEVITY OF RATS: ROLE OF HYPER INSULINEMIA

Investigators : D.Sreeramulu, T.Prasanna Krishna and M.Raghunath

Duration : 2 years

Date of completion : March 2004

Calorie restriction is known to increase longevity. However, the lack of any beneficial effect of calorie restriction on ageing among Indians is thought to be due to the associated

deficiency of micro-nutrients in traditional Indian diets. In our earlier studies in 24 month old rats, diet restriction (30 % of RDA) along with micro-nutrient sufficiency (100 % RDA) appeared beneficial in reducing free radical damage (Annual report 2001- 2002). However, reduction in oxidative stress was not statistically significant probably because the rats used in the study were very old and the aged rats might not have responded to dietary intervention.

It was recently hypothesized that oxidative stress / free radicals manifest their effects on ageing through modulation of hyper-insulinemia. Indeed, this hypothesis has been used as a metabolic explanation for the life prolonging effects of calorie restriction. Therefore in the present study, the effect of diet restriction with / without associated restriction of micro-nutrients was evaluated on the insulin and glycemic status of the animal to validate / negate the above hypothesis, in addition to determining the effect on oxidative stress and other age-related parameters.

OBJECTIVES

1. To assess the effect of diet restriction along with micro-nutrient (MN) sufficiency on the antioxidant status and / or longevity.
2. To determine the age at which dietary intervention (i.e. diet restriction + micro-nutrient sufficiency) is useful in improving the longevity and / or antioxidant status and
3. To validate the role of hyper-insulinemia in diet restriction and / or micro-nutrient supplementation on the modulation of changes in oxidative stress / longevity.

WNIN rats (twenty four each of 6,12 &18 month of age in Table 9) of both sexes were divided into three groups and fed different diets for six months as follows.

Table 9.

Age of the Rat (months)	Group 1 / Diet 1		Group 2 / Diet 2		Group 3 / Diet 3	
	Calories	Micro nutrients	Calories	Micro nutrients	Calories	Micro nutrients
6	100*	100	70	50	70	100
12	100	100	70	50	70	100
18	100	100	70	50	70	100

* Values given are as % of RDA

During the experimental feeding period of six months, body weight and haemoglobin level were determined at monthly intervals. Blood was drawn at baseline , 3 and 6 months of feeding and analysed for antioxidant enzymes, glucose and insulin levels. At the end of six months of feeding, oral glucose tolerance test (OGTT) was conducted in at least six animals per group. Animals were sacrificed after six months of feeding and liver, kidney, heart and brain were collected to assess the tissue oxidative stress / antioxidant status.

RESULTS

The salient observations of the study are

1. At baseline, there were no significant differences among the three groups of rats of each age group (6,12 and 18 months) in any of the parameter studied (Table 10).

Table 10. Some plasma Biochemical indices in WNIN rats of different ages (Baseline data)

Age of the rats (months)	Diet/ Group	Fasting glucose mg/dl	Fasting insulin µu/ml	HOMA IR	FRAP µmol/ml	TBARS nmoles/ml
6	1	74.6±3.43	65.5±10.38	12.2±2.13	37.3±6.42	13.1±1.36
	2	68.9±3.11	90.1±10.68	15.5±2.27	69.5±3.48	9.8±1.22
	3	81.8±.4.25	91.2±21.78	19.4±6.00	94.2±2.73	7.8±1.28
12	1	64.8±3.11	83.5±10.52	13.3±1.90	34.8±2.34	12.6±1.87
	2	72.1±3.98	133.5±10.36	23.4±1.30	59.0±3.71	11.2±1.38
	3	82.5±4.05	108.2±18.23	22.8±5.10	55.2±11.35	6.6±0.76
18	1	59.1±2.98	75.0±5.75	11.0±1.06	34.4±2.57	14.7±2.11
	2	77.9.±5.77	90.3±20.15	17.2±3.80	74.1±13.00	10.3±1.81
	3	80.8±1.85	105.4±20.93	20.6±4.18	56.5±10.18	7.0±0.86

® Values given are Mean±SE, n=6
® None of the parameters different between groups in rats of comparable age.

1. In rats of a given age, there were no significant differences among the three groups in food intake and body weight at any of the three time points (baseline, 90 days and 180 days).
2. At 90 days of feeding, there was an increase in fasting insulin and HOMA IR index in rats of all ages fed diet 2 (calorie & MN restriction) compared to diet 1 (control) and the effect was mitigated by diet 3 (calorie restriction & MN sufficiency). However none of the rats showed impaired OGT.
3. At this time point (90 days), diet 2 fed rats of all ages showed no significant changes in oxidative stress (TBARS) or antioxidant status (FRAP) compared to controls. Nevertheless, diet 3 improved antioxidant status and decreased oxidative stress in general.
4. At 180 days of feeding, in rats of all ages feeding diet 2 or diet 3 had in general, no significant effect on fasting insulin (except 18 month old rats on control diet which showed an increase of around 35 %). Fasting blood glucose was in general higher in these rats compared to controls (except 6 month old rats on diet 2) resulting in slight but not significant increase in HOMA IR index. Despite the slight alterations in these parameters all rats showed normal OGT.
5. In 6 and 18 months old rats fed diet 2 or 3, there was no significant effect on TBARS or FRAP at 180 days of feeding (but for the significant decrease in TBARS in 18 months old rats fed diet 3). On the other hand it was interesting that in 12-month-old rats at this time point of feeding, both diet 2 & 3 significantly increased FRAP. It was however puzzling that rats fed diet 3 but not diet 2 had significantly higher TBARS value at this time point.
6. In 12 month old rats, erythrocyte catalase and GSHPx activities were significantly higher at 180 days of diet 3 feeding (1.54 units / mg protein and 40.0 U NADPH oxidized / mg protein)
7. In 18 months old rats activities of GSHPx and SOD were significantly (26.11 U NADPH oxidized / mg protein and 1.31 units / mg protein) lower with diet 3, both at 90 and 180 days of feeding. The protective effect of diet 3 is not seen in 18 months old rats.
8. The oxidative stress in different tissues at the end of experimental feeding period indicate that in rats of 6 and 12 months of age, levels of TBARS were the least in the animals fed calorie restricted MN sufficient diet. However, calorie restriction along with that of MNs did not appear to affect this parameter compared to controls. On the other hand in 18-

month-old rats, calorie restriction along with that of MNs appeared to decrease TBARS surprisingly and feeding calorie restricted MN sufficient diets did not appear to have any significant effect in general. This discrepancy could be due to the old age of the rats as we observed earlier in 24-month-old rats.

9. The activity in the erythrocytes, of antioxidant enzymes: SOD, Catalase and GSHPx showed wide variation in rats of all ages studied, at baseline as well as at 3 and 6 months of feeding different diets. Further, neither calorie and MN restriction nor calorie restriction with MN sufficiency, showed any consistent or significant effect on the activity of any of these enzymes at any of the time point tested, in rats of any age studied.

That at 90 days of feeding, restriction of dietary micronutrients and calories (diet 2) but not calorie restriction alone (diet 3) had adverse effects on fasting insulin and HOMA IR index appears to stress the importance of MN in modulating hyperinsulinemia / insulin resistance. The improvement in antioxidant status / decrease in oxidative stress observed in rats fed diet 3 but not diet 2, not only seem to validate the role of hyper-insulinemia in modulating oxidative stress but also the role of MN status in this modulation.

However, the finding that similar effects on fasting insulin, HOMA IR, antioxidant status and oxidative stress were not observed in rats at 180 days of feeding appears to suggest the transient nature of the effect of MNs, vis a vis the parameters referred above.

That rats of 6 and 12 months of age but not those 18 month old, showed comparable effects of the calorie restriction with / without the concurrent MN restriction, considered together with the transient nature of the effect, 6 – 12 months appears to be the appropriate age at which MN supplementation along with calorie restriction may be beneficial in improving the antioxidant status and checking hyper-insulinemia in the rats.

Overall the results indicate the beneficial effect of calorie restriction along with MN sufficiency in protecting the animal against oxidative stress and hyperinsulinemia.

Table 11. Effect of caloric restriction with/without micronutrient restriction for three months on some plasma biochemical indices in WNIN rats of different ages

Age of the rats (months)	Diet/ Group	Fasting glucose mg/dl	Fasting insulin µu/ml	HOMA IR	FRAP µmol/ml	TBARS nmoles/ml
6	1	81.1±5.04	75.0±28.87 [a]	16.2±7.48 [a]	24.7±5.87 [a]	13.7±1.67
	2	86.4±2.21	100.3±8.85 [b]	21.3±1.84 [b]	11.7±1.48 [a]	9.3±0.78
	3	83.5±3.34	67.3±5.48 [a]	13.9±1.41 [c]	61.1±6.38 [b]	12.9±2.27
12	1	74.5±6.04 [a]	60.3±9.95	11.4±2.44 [a]	14.5±0.95 [a]	9.7±1.08 [a]
	2	114.4±3.64 [b]	85.3±8.49	24.0±2.32 [b]	17.5±5.26 [a]	10.8±1.11 [a]
	3	94.6±4.08 [c]	60.3±5.49	14.2±1.69 [a]	74.4±10.40 [b]	6.6±0.64 [b]
18	1	67.4±2.98 [a]	70.3±11.32	11.8±1.93 [a]	28.3±7.18 [a]	11.7±1.53
	2	106.±1.07 [b]	94.3±15.93	24.9±4.15 [b]	17.2±1.0 [a]	8.1±1.29
	3	86.0±3.22 [c]	59.8±5.18	12.6±0.93 [a]	71.3±6.5 [b]	8.4±0.79

® Values given are Mean±SE, n=6

® Values bearing different superscripts in a given column, for rats of similar age, significantly different by ANOVA/parametric or nonparametric test.

Table 12. Effect of caloric restriction with/without micronutrient restriction for six months on some plasma biochemical indices of WNIN rats of different ages

Age of the rats (months)	Diet	Fasting glucose mg/dl	Fasting insulin µu/ml	HOMA IR	FRAP µmol/ml	TBARS nmoles/ml
6	1	69.1±2.27[a]	77.3±12.38	13.4±2.54	36.9±6.53	6.3±1.68
	2	70.1±2.11[a]	71.3±9.53	12.4±1.81	60.1±4.80	7.5±1.19
	3	96.8±6.22[b]	73.5±7.07	18.0±2.64	46.6±6.07	8.0±0.84
12	1	76.7±1.49[a]	64.2±5.61	12.2±1.26[a]	26.9±5.83[a]	8.4±0.46[a]
	2	90.1±5.66[b]	62.5±6.40	13.8.0±1.46[a]	54.7±7.49[b]	7.4.8±0.66[a]
	3	97.6±6.17[b]	78.3±8.50	18.7±2.0[b]	61.3±3.39[b]	14.2±0.88[b]
18	1	75.6±0.86[a]	99.6±11.98	18.8±2.23[a]	52.5±3.97	11.5±1.20[a]
	2	97.4±8.00[b]	67.8±3.52	16.0±0.93[b]	46.7±8.71	9.0±0.80[ab]
	3	102.4±9.37[b]	67.4±5.94	16.8±1.53[b]	59.0±5.21	5.8±0.30[b]

® Values given are Mean±SE, n=6
® Values bearing different superscripts in a given column, for rats of similar age, significantly different by ANOVA/parametric or nonparametric test.

Table 13. Tissue TBARs (n moles/ mg protein) in WNIN rats of different ages fed different diets for six months

Age of the rats	Diet	Liver	Kidney	Heart	Brain
6	1	1.64±0.56	2.47±0.34	1.48±0.26	3.03±0.66
	2	1.87±0.37	2.24±0.40	1.55±0.41	2.54±0.56
	3	1.81±0.13	1.78±0.47	1.29±0.34	1.66*±0.27
12	1	2.57±0.27	1.93±0.08	1.57±0.42	3.08±0.85
	2	1.94±0.21	1.80±0.55	1.39±0.26	2.03±0.27
	3	1.61*±0.25	1.66±0.17	1.03*±0.21	1.29*±0.80
18	1	2.24±0.21	2.24±0.91	1.06±0.14	2.24±0.26
	2	1.61±0.24	2.06±0.42	0.95±0.51	2.05±0.54
	3	1.77±0.07	1.96±0.48	0.89±0.53	2.54±0.31

Table 14. Erythrocyte antioxidant enzymes in WNIN rats of different ages fed different diets for six months

Parameter	Age	Basal			90 days			180 days		
		Diet			Diet			Diet		
		1	2	3	1	2	3	1	2	3
Catalase U / mg prot	6	1.56± 0.30	1.77± 0.64	2.04± 0.90	1.50± 0.24	1.26± 0.38	1.58± 0.30	1.49± 0.43	1.53± 0.22	1.39± 0.31
	12	2.07± 0.97	1.70± 0.41	1.66± 0.41	1.65± 0.64	1.71± 0.29	1.60± 0.34	1.01± 0.36	0.95± 0.35	1.54± 0.36
	18	1.93± 0.19	1.47± 0.29	1.63± 0.41	1.57± 0.26	1.72± 0.45	0.92± 0.28	1.33± 0.27	1.29± 0.22	1.50± 0.12
GSH Px U of NADPH Oxi/min/mg pr	6	36.46 ±5.1	32.63 ±7.3	32.50 ±12.1	47.44 ±5.18	31.76 ±4.72	42.83 ±5.8	32.68 ±14.8	41.95 ±8.05	32.50 ±17.1
	12	39.92 ±12.9	39.01 ±5.2	52.78 ±4.9	31.15 ±4.8	34.63 ±8.2	36.6± 11.2	25.99 ±11.4	20.51 ±8.5	40.00 ±12.5
	18	45.31 ±14.7	32.59 ±10.8	40.30 ±11.5	21.04 ±6.3	34.68 ±13.2	38.05 ±10.2	30.98 ±4.4	23.40 ±4.5	26.11 ±2.4
SOD U/mgprotein	6	1.61± 0.15	1.56± 0.26	1.63 ±0.05	1.31± 0.23	1.21± 0.16	1.51± 0.32	1.11± 0.314	1.38± 0.16	1.21± 0.09
	12	1.52± 0.13	1.45± 0.12	1.63± 0.22	1.43± 0.16	1.58± 0.11	1.52± 0.07	1.36± 0.32	1.32± 0.22	1.60± 0.28
	18	1.78± 0.16	1.58± 0.09	1.33± 0.19	1.54± 0.06	1.10± 0.10	1.09± 0.06	1.43± 0.29	1.06± 0.05	1.31± 0.09

8. INHIBITION OF ALDOSE REDUCTASE BY TANNOID PRINCIPLES OF *EMBLICA OFFICINALIS* AND PREVENTION OF SUGAR CATARACT IN LENS ORGAN CULTURE

Investigators : P. Suryanarayana and G. Bhanuprakash Reddy

Duration : 2 Years

Date of completion : November 2003

BACKGROUND

Diabetes has been considered to be one of the major risk factors of cataract. During hyperglycemia the cellular levels of glucose greatly increase in tissues where glucose entry is independent of insulin. In these tissues, which include lens, retina, kidney and peripheral nerves, this excess glucose is metabolized via an accessory pathway known as the polyol pathway. Aldose reductase (AR: EC 1.1.1.21**)** is the rate limiting enzyme of the polyol pathway. AR catalyzes the conversion of glucose to sorbitol. Reduction of excess glucose to the osmolyte, sorbitol, leads to osmotic swelling, changes in membrane permeability and subsequent cataract formation. Therefore, aldose reductase (AR) has been a drug target because of its involvement in the development of secondary complications

of diabetes including cataract. Though, numerous synthetic AR inhibitors (ARI) have been tested and shown to inhibit the enzyme, clinically, synthetic ARIs have not been very successful. Thus, evaluating natural sources for ARI potential may lead to the development of safer and more effective agents against diabetes complications. In the present study we have assessed the inhibition of AR by the constituents of *Emblica officinalis* in vitro and in lens organ culture as a part of our program on screening of plant/ spice sources for ARI and testing their anticataractogenic potential.

METHODOLOGY

Crude aldose reductase was prepared from rat lens. Lenses were dissected from WNIN rats and homogenized in 10 volumes of 100 mM potassium phosphate buffer pH 6.2. The homogenate was centrifuged at 15,000g for 30 min at 4°C and the resulting supernatant was used as the source of AR. Recombinant human AR was expressed in bacterial cultures. Enzyme from expression cultures was extracted and purified by affinity chromatography over AffiGel Blue. Fresh fruits of *Emblica* were obtained and freeze-dried. Dried material was powdered and a water extract was prepared by stirring at room temperature for 3 h. Insoluble material was removed by centrifugation followed by filtration. Tannoid principles from amla were isolated according to Ghosal et al (1996) and obtained in the form of a standardized mixture of emblicanin A, emblicanin B, punigluconin and pedunculagin from Indian Herbs Research & Supply Co. (Saharanpur, India). Aqeous extract of *Emblica officinalis* and its major constituent tannoids were tested for inhibition against both rat lens and purified recombinant human AR. AR activity was assayed spectrophotometrically in the absence and presence of Emblica components. ARI potential of isolated tannoids of *E. officinalis* was investigated against osmotic stress in rat lens organ culture. Each isolated lens was incubated in 2 ml of modified TC-199 medium with antibiotics at 37°C under 95% air and 5% CO_2 with and without 55 mM glucose for a period of 18 h (for sorbitol estimation) or for 4 days (for lens morphology). Medium was changed every 48 h. When added to medium, stock of mixture of tannoids of Emblica was prepared in water and filtered through 0.2 µm filters. The sorbitol content of the lenses was measured by enzymatic method.

RESULTS

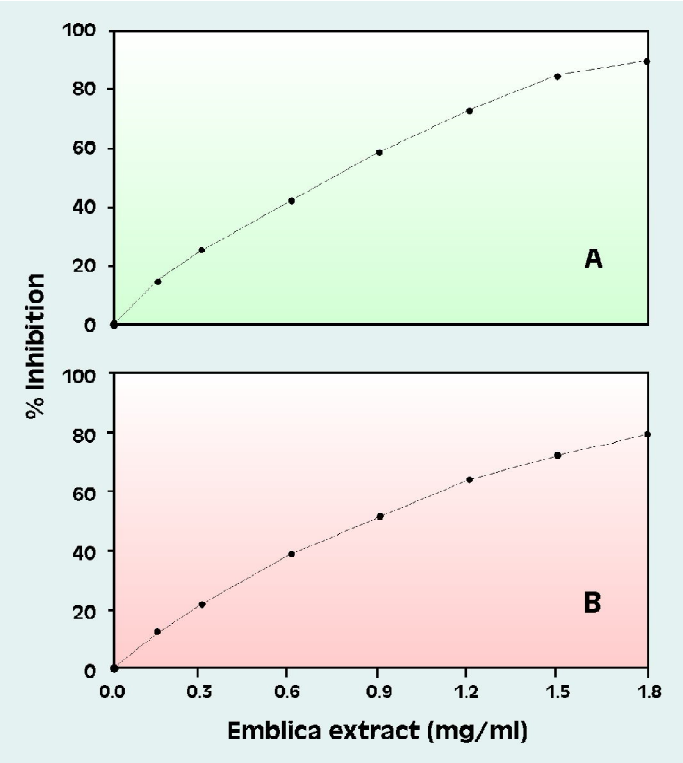
(i) Aquesous extract of *E. officinalis* inhibited rat lens AR with IC_{50} values 0.72 mg/ml (Fig 12A).

(ii) However, rat lens is known to have highest AR activity compared to other species. Moreover, the other constituents in the crude preparation of rat lens AR could complicate the results of AR inhibition by amla. Thus the relevance of inhibition of rat lens AR by *E.officinalis* may have limited application to human diabetic cataract.

(iii) Therefore, we have also assessed the inhibitory potential of *Emblica* against purified human recombinant AR expressed in *E. coli*. Interestingly aqueous extract of *Emblica* inhibited human recombinant AR to the same extent as that of rat lens AR with an IC_{50} value of 0.88 mg/ml (Fig.12B).

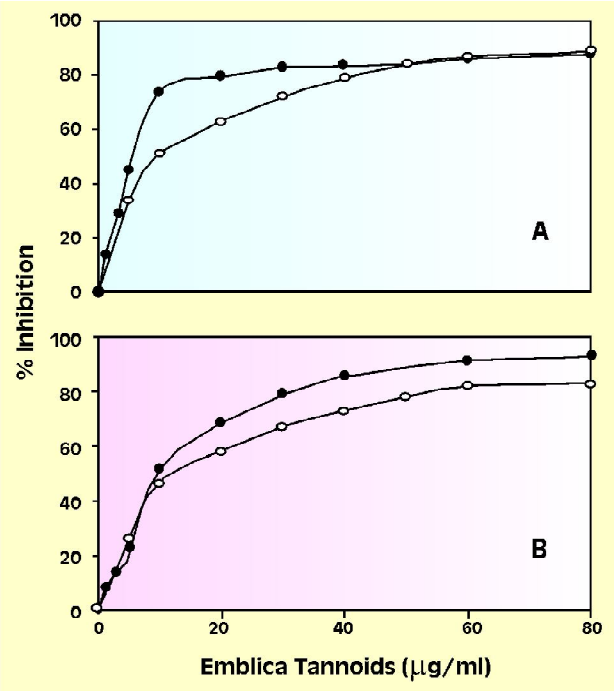
(iv) Since amla is a rich source of vitamin C (ascorbic acid), it is believed that the major constituent responsible for most of the biological actions of amla is vitamin C. However, vitamin C did not inhibit AR at concentrations as high as 5 mM, thus suggesting that AR inhibition by *Emblica* is due to some other constituents other than vitamin C.

Figure 12. Inhibition of rat lens AR (A) and human recombinant AR (B) by aqueous extract of E. officinalis



(v) Further, we demonstrate that the hydrolysable tannoids of *E. officinalis* were responsible for AR inhibition, as enriched tannoids of *E. officinalis* exhibited remarkable inhibition against both rat lens and human AR with IC_{50} of 6 and 10 mg/ml respectively (Fig. 13). The inhibition of AR by *Emblica* tannoids is 100 times higher than its aqueous extract and comparable or better than quercetin, a known AR inhibitor.

Figure 13. Representative graph of inhibition of rat lens AR (A) and human recombinant AR (B) by isolated tannoids of *E. officinalis* (solid circles) and quercetin (open circles)



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(vi) To understand the significance of in vitro inhibition of AR by amla, the effect of isolated tannoids against osmotic stress were investigated in lens organ culture. Incubation of lenses with 55 mM glucose resulted in increased production of sorbitol with increased AR activity compared to control lenses (Table 1). Remarkably, activation of AR and increased sorbitol levels due to hyperglycemic stress was prevented when lenses were incubated with 55 mM glucose in the presence of 50 µg/ml amla tannoids in the medium (Table 15).

Table 15. Effect of tannoid mixture of *Emblica* on aldose reductase activity and sorbitol levels in rat lens incubated in the absence and presence of 55 mM glucose for 18 h.

Group	AR activity[1]	Sorbitol[2]
Control	26.4 ± 2.45	19.4 ± 1.74
Glucose 55 mM	36.2 ± 3.51*	83.1 ± 9.13*
Glucose 55 mM + 50 µg/ml Tannoid mixture	29.8 ± 2.82	61.2 ± 10.27*#

Values are mean ± S. D. of three independent experiments.
[1] µmoles NADPH oxidized/h/100 mg protein; [2] nmoles/ 100 mg lens.
* Statistically significant from control group (P< 0.05)
# Statistically significant from Glucose 55 mM group (P< 0.05)

(i) While the lens incubated with glucose 55 mM for 4 days became cloudy and lost it transparency, the morphology of the lens incubated with 55 mM along with 50 µg/ml of tannoid mixture of amla appears similar to control lenses even after 4 days.

CONCLUSIONS

The results indicate that the in vitro inhibition of AR by *Emblica* is due to its tannoids. The inhibition of AR by Emblica tannoids could be correlated with in vivo conditions as it prevented changes associated with AR activation in lens organ culture. These results indicate that tannoids of *E. officinalis* are potent inhibitors of AR and suggest that exploring the therapeutic value of natural ingredients that people can incorporate into everyday life may be an effective approach in the management of diabetic complications. Studies are planned to investigate the potential of Emblica tannoids to prevent the cataract in experimental animals.

9. EFFECT OF NON-ENZYMATIC BROWNING ON THE MOLECULAR CHAPERONE-LIKE FUNCTION OF a-CRYSTALLIN

Investigators : M.Satish Kumar, P. Yadagiri Reddy and G. Bhanuprakash Reddy

Duration : 3 years

Date of completion : January 2004

BACKGROUND

Eye lens a-crystallin undergoes numerous posttranslational modifications (PTM) such as phosphorylation, glycation, oxidation, C-terminal truncation, deamidation during

aging and in cataract. Some of these PTM may influence the chaperone like function of the protein. Some of the above mentioned PTM are likely to be accelerated in diabetic/ hyperglycemic conditions particularly the non-enzymatic glycation. Several diabetic complications including cataract are thought to be the result of accumulation of advanced glycation endproducts (AGE) generated from modification of proteins by different glycating agents. AGE formation alters structural and functional properties of many proteins, which are implicated in the diabetic and uremic complications. Reactive dicarbonyl compounds, methylglyoxal (MGO) and glyoxal (GO) are formed from triose phosphates and also upon autooxidation of reducing sugars. Methylglyoxal (MGO), a major dicarbonyl compound, is present in high concentrations in lens compared to plasma or any other tissue and its levels increase several fold during diabetes. Compared to other potential glycating agents, MGO has very high affinity for proteins and is known to react with Arg, Lys, His and Cys residues forming AGE. Therefore, we have investigated the effect of MGO-modification on a-crystallin chaperone-like function and its physiological significance.

METHODOLOGY

a-Crystallin was isolated from bovine lenses by gel filtration (Sephacryl S-300HR) and incubated with various concentration of methylglyoxal (MGO) and glyoxal (GO) for 3 days at 37°C under sterile conditions. At the end, protein was dialysed extensively to remove unbound MGO and GO. Extent of modification was monitored by AGE fluorescence (ex 370 nm and em 400-500 nm) and argpyrimidine fluorescence (ex 320 nm; em 350-450 nm). Tryptophan fluorescence was monitored at ex 295 nm and em 300-400 nm. Chaperone activity was assessed by measuring the ability of a-crystallin to suppress the aggregation of insulin (induced by DTT) and citrate synthase & $ß_L$-crystallin (induced by heat). The potential of native and modified a-crystallin to prevent the inactivation of enzymes (glucose-6-phosphate dehydrogenase, G6PD and restriction enzyme, Sma I) due to heat or UVB irradiation was assessed. Hydrophobicity was measured by ANS binding. Secondary and tertiary structure was assessed by far- and near-UV CD spectra. Oligomeric size was estimated by HPLC using TSK G4000 SW gel filtration column. Non-specific proteins such as thyroglobulin, catalase, BSA and lysozyme were also modified with MGO to understand the specific effect of MGO on a-crystallin.

RESULTS

(i) Increased AGE fluorescence in a dose-dependent manner indicates formation of AGE-modified a-crystallin upon incubation with MGO and GO (Fig. 14A).

(ii) Synchronous fluorescence spectra of MGO/ GO treated a-crystallin indicate the presence of chromophores with excitation maxima at higher wavelength (>300 nm) and decrease in tryptophan absorption (Fig.14B)

(iii) AGE-modifed a-crystallin displayed increased chaperone-like activity against both DTT-induced aggregation of insulin and heat-induced aggregation of citrate synthase (Fig. 15).

(iv) Similarly enhanced chaperone-like activity was observed with abrin and $ß_L$-aggregations assays.

(v) In an attempt to understand the mechanism of enhanced chaperone activity of MG-modified a-crystallin, hydrophobicity, oligomeric size and secondary/tertiary structure was assessed after modification.

(vi) Though, hydrophobic patches on the surface of a-crystallin are thought to be responsible for chaperone activity, incubation with MGO and GO resulted in decreased hydrophobicity and fails to explain the increased chaperone activity.

(vii) There was a decrease or complete loss of secondary structure and altered tertiary structure of a-crystallin due to MGO/GO-induced modification.

(viii) Interestingly, the oligomeric size of a-crystallin was increased (to 2000 kDa) upon modification (Fig. 16). This prompted us to investigate whether such a non-specific aggregate resulting upon MGO-modification was responsible for enhanced chaperone function.

(ix) Therefore, we have modified several unrelated proteins, studied their aggregate size and chaperone-like ability. Similar to MGO-modified a-crystallin, MGO-modification of thyroglobulin, catalase showed chaperone-like function but not BSA and lysozyme. The chaperone-like activity of MGO-modified thyroglobulin and catalase was associated with the formation of high molecular weight aggregates similar to MGO-modified a-crystallin.

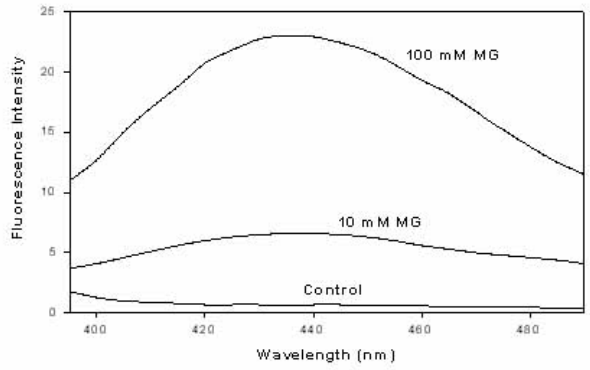
(x) Thus, our findings suggest that enhanced chaperone-like function of MGO-modified a-crystallin may be due to large HMW aggregate of non-specific nature and therefore the results of increased chaperone activity observed with in vitro aggregation assays may not have any relevance *in vivo*.

(xi) Hence, we have also assessed the potential of MGO-modified crystallin using non-aggregations assays. MGO-modified a-crystallin was less effective in protecting G6PD or SmaI against heat-induced inactivation compared to native a-crystallin (Fig. 17)

CONCLUSIONS

The observation that modified a-crystallin showed enhanced chaperone-like activity in aggregation assays but not in functional assays (where it showed decreased chaperone-like activity), suggest two possibilities. Either the mechanism of chaperone-like function may be different in different assays or the aggregation assays may not be an appropriate measure of chaperone activity in different circumstances (*i.e.* advanced glycation in the present study). However, it ought to be realized that glycation (AGE formation) has been implicated in the pathogenesis of various pathologic complications including cataract. Furthermore, if the enhanced chaperone-like activity of a-crystallin due to glycation is considered to be a true observation, it would be difficult to reconcile the fact that glycation-induced cataract could be a result of increased chaperone-like activity of a-crystallin. Hence further investigations are required to understand the effect of MGO on lens transparency and chaperone activity.

Figure 14. AGE-fluorescence (A) and synchronous fluorescence (B) of a-crystallin upon incubation with methylglyoxal for 3 days



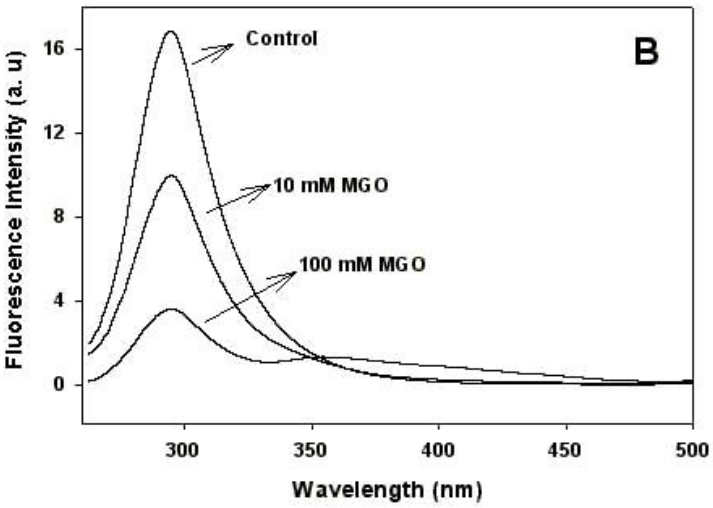
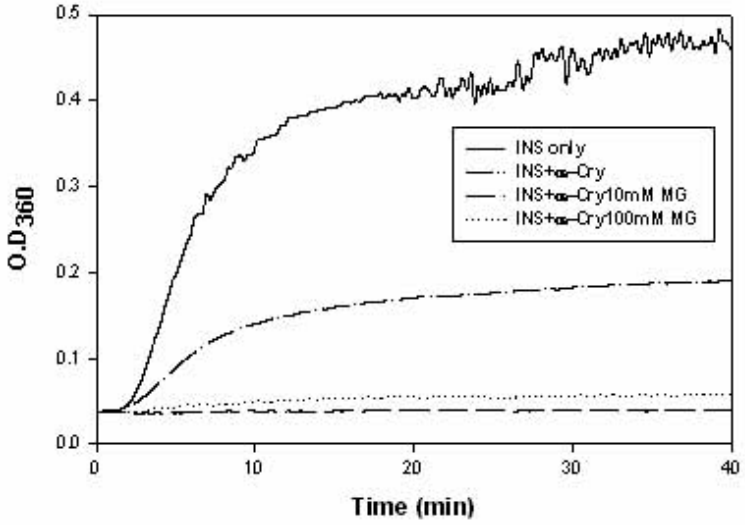


Figure 15. Chaperone activity of MG-modified α-crystallin against (A) DTT-induced aggregation of insulin and (B) heat-induced aggregation of citrate synthase



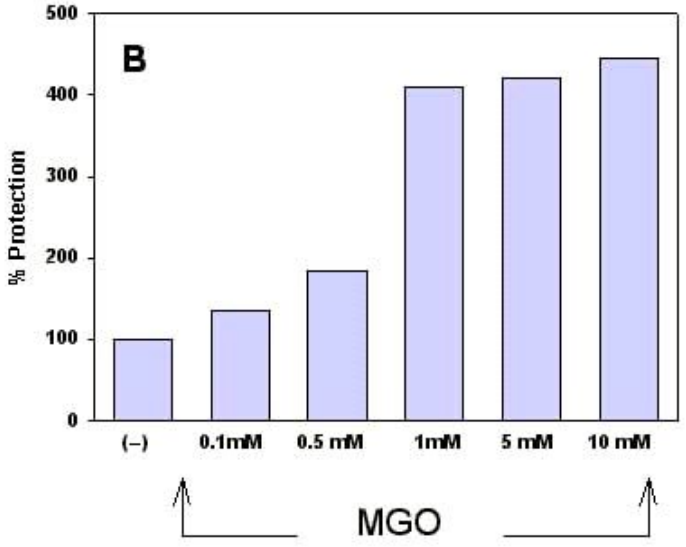


Figure 16. Effect of MGO modification on oligomeric size of α-crystallin (TSK G4000SW size exclusion HPLC column)

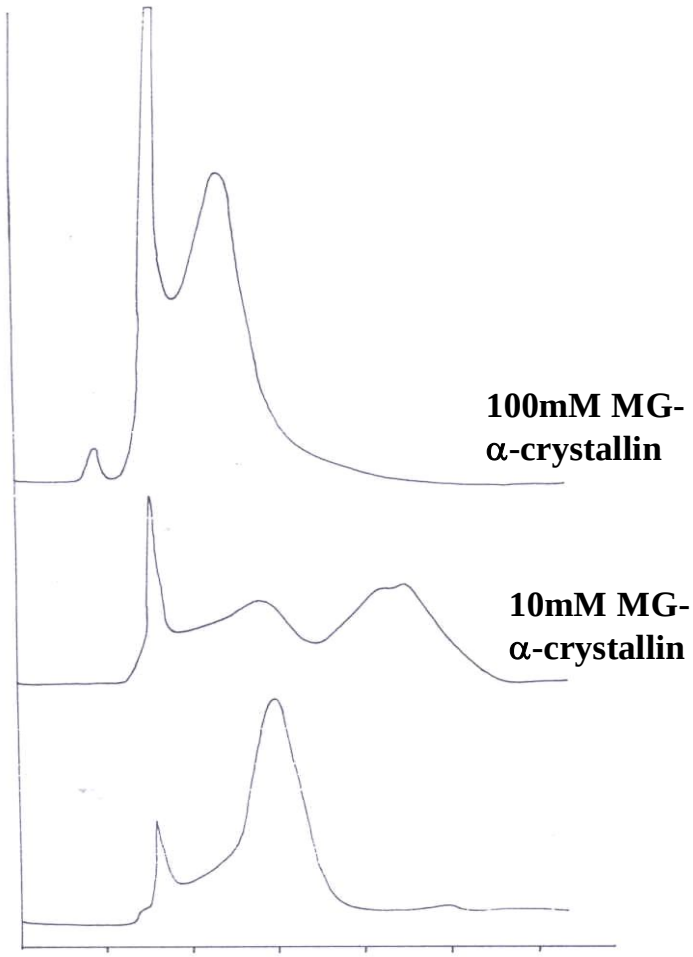
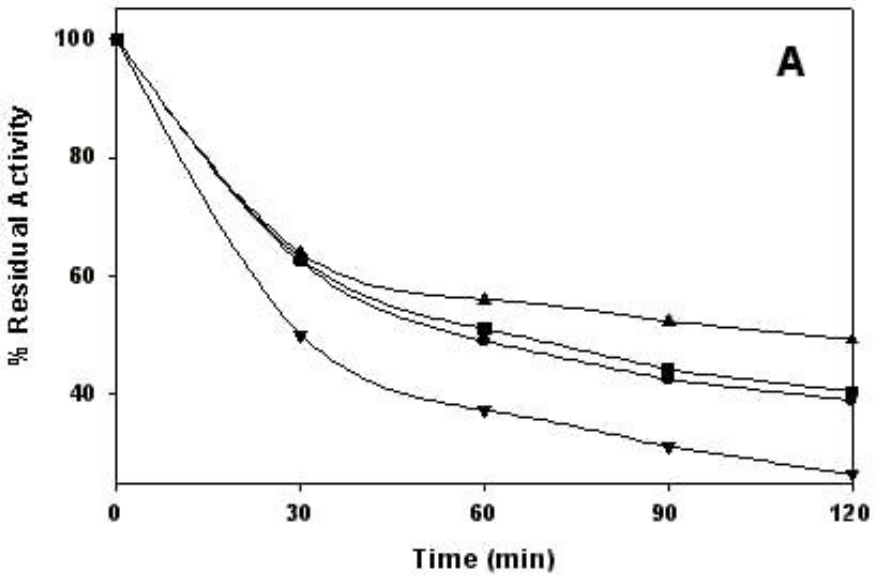
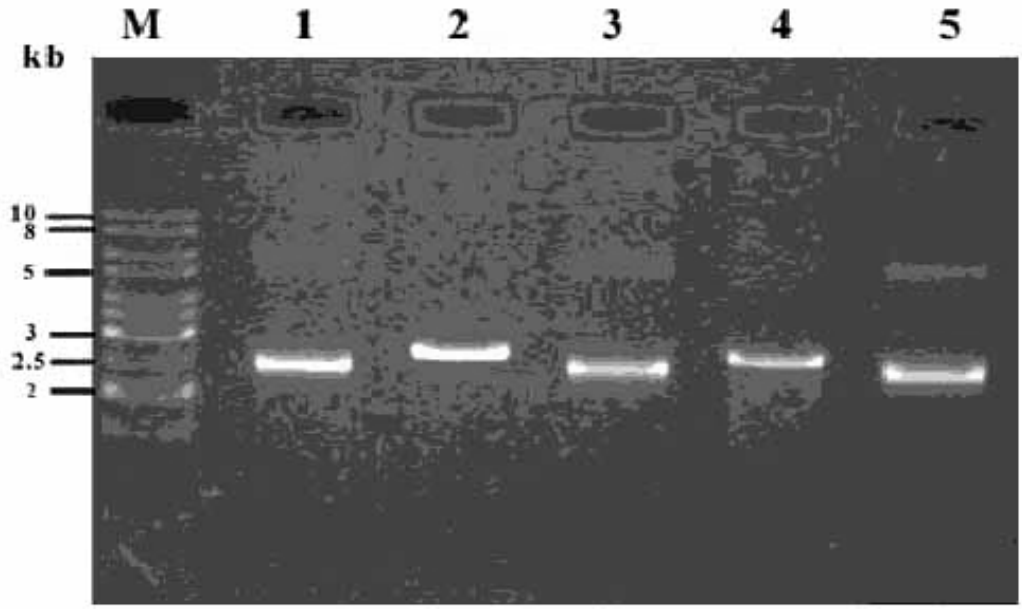


Figure 17. Chaperone activity of MGO-modified α-crystallin (A) Heat-induced inactivation of G6PD as a function of time in the absence (?) or in the presence of native (?), 10 mM (¦)and 100 mM (?) MGO-modified α-crystallin. (B) Protection of restriction enzyme, Sma I from heat inactivation by native and modified α-crystallin. *Lane 1*- DNA standards, *Lane 2*- uncut plasmid, *Lane 3-6*- plasmid digested with native Sma I, heat inactivated Sma I in the presence of native and MGO-modified α-crystallin, respectively





10. CHARACTERIZATION OF SUBSTRATE BINDING REGION ON α-CRYSTALLIN WIH RESPECT TO MOLECULAR CHAPERONE-LIKE FUNCTION

Investigator : P. Yadagiri Reddy and G. Bhanuprakash Reddy

Duration : 2 years

Date of completion : Februray 2004

BACKGROUND

α-Crystallin, the small heat shock protein (sHSP) present in the eye lens, is constituted of two closely related sub units, αA and αB, which form an aggregate of 800 kDa. Both αA and αB-crystallins have recently been shown to function like molecular chaperones. Current interest in our laboratory is focused to understand the effect of various posttranslational modifications (PTM) in particular glycation, oxidation and C-terminal truncation on the chaperone potential and modulation of these PTM by dietary factors. We have shown earlier that C-terminally truncated αA-crystallin has reduced chaperone function probably due to impaired complex formation between the substrate and the chaperone (Ann Report 2001-02) and suggested that extreme C-terminal tail of αA-crystallin may be involved in the chaperone function. Our earlier studies with single tryptophan mutants also suggest that C-terminal region is crucial for chaperone activity (*Invest. Ophthalmol. Vis. Sci.* 41, Suppl 3982, 2000). Studies carried out elsewhere implicated other regions for chaperone-like function in addition to C-terminal region. Of these, particular interest is on chaperone regions, sequence 70-88 and sequence 75-103 respectively in αA- and αB-crystallins. Indeed synthetic peptides corresponding to these regions are reported to function-like chaperones against insulin aggregation and describe them as mini chaperones of αA- and αB-crystallins. However, we have reported that, being very small in size insulin aggregation suffers some disadvantage and characterized a new model substrate, abrin, which is devoid of the disadvantages of insulin (*FEBS Letts*. 522, 59-64). Therefore studies were planned to characterize the substrate binding regions of molecular chaperone of α-crystallin using the three different synthetic peptides corresponding to C-terminal region of αA- and mini-chaperone regions of αA- and αB-crystallins.

METHODOLOGY

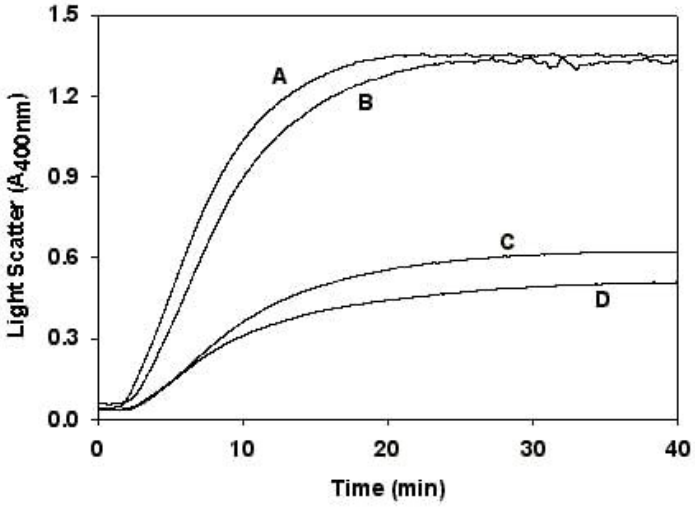
Synthetic peptide corresponding to C-terminal region of αA- (extreme 19 residues-AERAIPVSREEKPTSAPSS) was synthesized by solid-phase peptide synthesis (in Prof. Surolias' lab, MBU, IISC, Bangalore) and mini-chaperones of αA- (70-88 region-DFVIFIDVKHFSPEDLTVK) and αB-crystallins (73-92 region- DRFSVNLDVKHFSPEELKVK) were gifted by Dr. K. K. Sharma (Mason Eye Institute, University of Missouri, USA). A peptide with unrelated sequence (AIHKLAHKLLKKLLRAVKKLANA), was synthesized by solid-phase peptide synthesis at IISc, was used as a control peptide.

Chaperone activity was assessed by measuring the ability of the above peptides to suppress the aggregation of insulin and abrin (induced by DTT) and to protect the glucose-6-phosphate dehydrogenase (G6PD) against UVB-induced inactivation using the methods described earlier (*Biochem. Biophys. Res. Commun.* 282, 712-716). Their chaperone activities were compared with that of the full length αA and αB-crystallins. To investigate the effect of glycation on these peptides, they were incubated with 5 mM methylglyoxal for 3 days at 37°C. Molecular size and chaperone activity of the modified peptides were assessed by gel filtration (HPLC) and aggregation assays.

RESULTS

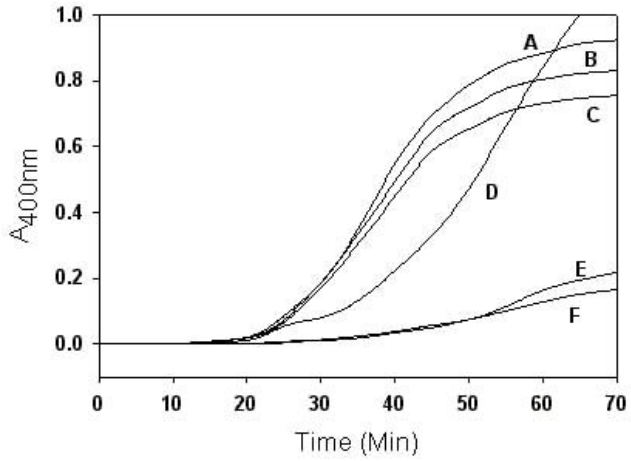
1) As reported earlier, mini-chaperones of A- and B-crystallins were able to suppress the aggregation of insulin. However, the C-terminal peptide of A-crystallin was unable to suppress insulin aggregation (Figure 18).

Figure 1: Chaperone-like activity of peptides of α-crystallin against DTT-induced aggregation of insulin. Insulin alone, 0.4 mg/ml (A), insulin + 50 µg/ml C-terminal peptide (B), insulin + 50 µg/ml mini αA peptide (C) and insulin + 50 µg/mlµg/ml min αB peptide (D). Online light scattering was measured at 400 nm.



Full length αA- and αB-crystallins were able to suppress abrin aggregation (90%) at 0.65 mg/ml. However neither of the two mini-chaperones nor the C-terminal peptide of αA-crystallin could to suppress the aggregation of abrin even at the 10 times greater molar concentrations of full length proteins (Figure 19).

Figure 2: Chaperone-like activity of peptides of α-crystallin against DTT-induced aggregation of abrin. Abrin alone, 0.6 mg/ml (A), abrin + mini αA peptide (B), abrin + mini αB peptide (C), abrin + C-terminal peptide (D), abrin + αA-crystallin (E) and abrin + αB-crystallin (F). Online light scattering was measured at 400 nm.



1) The mini-chaperones were not effective in protecting G6PD against UVB-induced inactivation even at 5mM concentrations.
2) The C-terminal peptide of αA-crystallin was also unable to protect G6PD whereas full length proteins were able to protect G6PD 90% at 0.15 µM concentration.
3) While this work was in progress, importance of another stretch of sequence in the N-terminal regions was reported elsewhere.
4) These results together suggest that, though some isolated regions may not directly exert the anti-aggregation potential, they may contribute to the structural stability, subunit interaction and higher order oligomeric assembly.
5) As the results with peptides indicate the importance of the oligomer of α-crystallin as the structural unit for the chaperone activity, rather than isolated regions of the protein, studies are planned to get insight into the nature of oligomer and mechanism of action by manipulating the oligomerization of α-crystallin.
6) It was found that upon non-enzymatic browning of α-crystallin by methylglyoxal led to increased oligomeric size and enhanced chaperone activity (Ann. Rep. 2003-04). However, incubation of the peptides with methylglyoxal could not induce any aggregate formation nor affected their chaperone activity against abrin aggregation..

CONCLUSIONS

Insulin-B chain is a very small peptide, whose aggregation could be suppressed by peptides corresponding to chaperone regions of αA- and αB-crystallins. But the same peptides were not able to suppress abrin aggregation. Most probably, this may be due to large size of abrin-B chain and that of mini chaperones were not big enough to hold the abrin B-chain in partially unfolded state. On the other hand C-terminal region may serve as solubilizer than a mini-chaperone, as it was not able suppress even insulin aggregation. Therefore, it appears that complete αA- and αB-crystallin sequences with specific oligomeric structures may be essential for chaperone-like function by providing a large surface area.

11. ANALYSIS OF TRIBAL FOODS (UNCULTIVATED) IN COLLABORATION WITH DECCAN DEVELOPMENT SOCIETY

Deccan Development Society with its Branch Krishi Vigyana Kendra an NGO is involved in keeping the biodiversity in the Zaheerabad area of Medak District. In the summer months, the people in the area (mostly tribals) consume a few wild and uncultivated greens. They had requested us to help them in getting some of those samples analysed.

In view of their nature as these are consumed by tribal population and to collaborate with the NGO, we have carried out the study to analyse these plant sources. They have also agreed to meet a part of the expenditure involved in the projects.

All the plants were identified botanically and analysed for vitamin C, carotenoids and B-carotene.

The results are given in the table.

RESULTS AND DISCUSSION

Results presented in the table showed that there are differences in the vitamin C content in 6 green leafy vegetables when they are grown in red soil. It is known that the

nutrient content depends directly on the soil nutrient content or fertility in general. With regard to total carotenoids and ß-carotene there are no major differences in the green leafy vegetables whether they are grown in black soil or red soil. Most of the foods are rich in carotenoids in general. The values for folic acid content of the foods is awaited.

Moisture, Vitamin C, Total and β-Carotene Content of Less Familiar Green Leafy Vegetables, Flowers and Root Grown in Red and Black Soil of Zaheerabad

Sl. No	Name of the sample	Type of soil	Moisture g/100g	Vitamin C mg/100g	Total carotenoids µg/g	β carotene µg/g
1.	**Sannapayala**	Red soil	92.63	47.43	68.97	22.86
		Black soil	92.95	60.16	64.26	24.70
2.	**Adavi soyakura**	Red soil	50.38	219.68	221.65	37.70
3.	**Athelli**	Red soil	87.96	64.75	232.75	64.54
		Black soil	81.39	91.53	239.87	64.61
4.	**Pittakura**	Red soil	41.80	296.03	297.45	49.04
		Black soil	44.88	198.46	346.21	110.02
5.	**Bankantikura**	Red soil	77.67	151.20	221.31	70.19
		Black soil	42.23	117.18	287.31	84.31
6.	**Chennagi**	Red soil	77.37	260.85	334.40	90.29
7.	**Yennadri**	Red soil	71.76	123.60	190.92	35.02
		Black soil	70.10	103.90	219.59	41.64
8.	**Uttareni**	Red soil	82.91	31.75	184.86	60.22
		Black soil	80.15	94.56	186.76	53.11
9.	**Gunugu**	Red soil	80.57	99.40	170.16	29.63
		Black soil	79.18	125.45	217.39	39.67
10.	**Buddakasha**	Red soil	84.34	135.48	247.41	63.40
		Black soil	76.29	114.10	202.24	31.28
11.	**Gorumadi**	Red soil	87.63	441.72	304.65	57.39
12.	**Tellagarjala**	Red soil	91.40	88.58	135.96	60.52
		Black soil	91.98	77.32	126.73	61.12
13.	**Thadakadobbudu**	Red soil	80.99	123.48	277.90	77.79
		Black soil	76.66	175.85	323.68	93.21
14.	**Jonnachenchali**	Red soil	79.77	127.42	191.06	53.60
		Black soil	71.86	101.45	182.80	64.38
15.	**Pullakura**	Red soil	82.29	84.24	169.72	50.80
		Black soil	41.32	249.59	476.01	113.08
16.	**Tagarancha**	Red soil	82.55	225.01	234.75	104.18
		Black soil	84.90	197.98	177.60	67.42
17.	**Yellakachevulakura**	Red soil	50.78	163.96	369.90	87.87
		Black soil	30.39	111.80	485.54	128.82
18.	**Angibingi**	Red soil	83.03	204.18	336.35	84.69
		Black soil	85.76	239.87	371.95	93.44
19.	**Pappukura**	Black soil	33.01	1045.52	503.57	94.04
20.	**Adavimenthakura**	Black soil	57.82	260.12	199.88	29.57
21.	**Tummikura**	Red soil	61.92	134.93	207.94	31.55
		Black soil	70.18	174.96	350.76	70.20
22.	**Gurumashi**	Red soil	72.42	391.16	509.04	148.91
		Black soil	75.76	161.27	424.57	98.36
23.	**Adavi ponnaganti alam**	Red soil	86.91	110.18	176.08	54.34
		Black soil	87.23	107.75	140.79	51.50
24.	**Nallakasha**	Red soil	75.45	257.70	402.0	139.19
		Black soil	85.30	113.41	208.75	56.74
25.	**Dooseri**	Red soil	74.91	232.17	232.45	43.05
26.	**Taliala**	Red soil	86.47	84.48	346.83	124.48
		Black soil	84.50	106.23	288.35	111.83
27.	**Gurumashi gadda**	Red soil	67.92	388.19	**Not detectable**	
		Black soil	71.36	263.36	**Not detectable**	

Values are average of duplicate determinations

IV. PATHOLOGY

1. EFFECT OF VITAMIN RESTRICTION AND SUPPLEMENTATION ON DRUG INDUCED APOPTOSIS OF RAT INTESTINAL MUCOSAL CELLS

Investigators : A. Vijaya Lakshmi, B. Sesikeran, P. Uday Kumar, Kalyanasundaram and M. Raghunath

Duration : 1 year

Date of completion : Sept. 2003

BACKGROUND

Most of the anticancer drugs are not target specific and can affect both normal and malignant cells, which contributes to the side effects observed during chemotherapy. Intestine and bone marrow are among the tissues, which get affected because of their high proliferation rates. In this regard any specific dietary intervention, that can enhance the drug sensitivity of the tumor cells and/or prevent its action on normal cells would be of great help in cancer treatment. With this aim and to validate our hypothesis that nutritional factors also modulate drug induced apoptosis, the effect of vitamin restriction (VR) and supplementation was studied. Cisplatin (CDDP) a chemotherapeutic drug was selected to induce apoptosis in the intestinal mucosal (IM) cells. Since there is no information on the dosage of cisplatin to be used in an experimental set up that would modulate only apoptosis without increase in mortality, the specific dose was standardized in control diet fed rats and 3.5mg of CDDP/kg body weight was found to be appropriate for this purpose. Since vitamin restriction *per se* induced intestinal mucosal cell apoptosis, the dosage of CDDP was fixed suitably by conducting preliminary studies in VR rats with different CDDP doses. It was observed that in VR rats, a weekly dose of 2.61mg of CDDP/kg body weight was best suited for this purpose. Based on the results obtained, it was hypothesized that vitamin supplementation to VR animals modulates the drug (cisplatin) induced oxidative stress and apoptosis in IM cells. An experiment to validate this hypothesis, in addition to deciphering the probable mechanisms of the effects, was done.

OBJECTIVES

1. To study the effect of 50% total vitamin restriction and vitamin supplementation on drug induced apoptosis of rat intestinal mucosal cells.
2. To look into the mechanisms involved in this alteration

METHODOLOGY

A total of 72, male Wistar NIN rats, 21 days old were divided into two groups; control and 50% vitamin restriction. These animals were fed on respective diets for 17 weeks and each group was sub divided into 6 groups, each having 6 animals. One of these subgroups, each from control and VR group, received saline and acted as vehicle control. The remaining 5 sub groups of control and VR rats received 2.61mg of cisplatin/kg bodyweight for three weeks at weekly intervals. Second subgroup received only cisplatin (CON + CIS), third subgroup received riboflavin (CON + CIS + RBFS) in addition, fourth subgroup folic acid (CON + CIS + FAS), fifth subgroup vitamin E (CON + CIS + VES), sixth subgroup multiple vitamin mixture (CON + CIS + TVS) supplemented diets for three weeks (18-20th

week) along with cisplatin. At the end of 20 weeks all the animals were sacrificed. Blood and vital organs were collected to check for toxicity and jejunum was collected to study apoptotic changes. Body weights and food intake were monitored at periodic intervals. Hemoglobin, RBC, WBC, platelet counts, plasma vitamin status and histopathological changes in vital organs were observed to assess the toxic effects of cisplatin with vitamin restriction and supplementation.

SALIENT FINDINGS

1. Cisplatin *per se* increased apoptotic rates, oxidative stress and decreased Bcl-2 expression in both control and vitamin restricted animals.
2. All three individual vitamin and multiple vitamin supplementation decreased oxidative stress, but it was only riboflavin and folic acid which decreased the apoptotic rates and increased Bcl-2 expression.
3. Riboflavin and folic acid supplementation protected the cells from cisplatin induced damage in both control and VR animals. Surprisingly vitamin E supplementation did not offer protection against cisplatin induced mucosal damage although it mitigated the oxidative stress.
4. Cisplatin increased p53 expression in control animals, but the protein levels were not detectable in VR animals, although VR induced greater apoptosis. Riboflavin supplementation further increased the p53 levels. Lack of any apparent correlation between p53 expression and cisplatin induced apoptosis could be due to the possibility that p53 may not be involved in cisplatin induced apoptosis, or the p53 that was detected in the present study was probably a mutant form (mutant forms are also known to cross react with p53 antibodies). The role of p53 in DNA damage induced apoptosis needs to be studied further.
5. The effects of vitamin supplementation were surprisingly different in control and vitamin restricted animals treated with cisplatin, with respect to food intake and body weights. They did not show any effect on these parameters in control rats, but when VR animals were supplemented with vitamins, further decrease of food intake and body weights were observed, an observation for which we have no plausible explanation at present.
6. Vitamin supplementation decreased platelet counts in VR animals, which could be due to increased apoptosis of stem cells in the bone marrow, whereas in control animals only vitamin E supplementation showed decreased platelet counts.
7. The plasma vitamin status too was decreased, probably because of the loss of absorptive cells and vitamin supplementation helped in improving the circulatory vitamin levels.
8. Mild interstitial nephritis was observed in control animals and this was more in vitamin restricted animals. Surprisingly, the severity of kidney damage increased further, with vitamin supplementation in both control and restricted animals.

From these findings it appears that the role of oxidative stress may albeit be marginal in cisplatin induced apoptosis, and it's toxicity appears to be mostly through DNA damage (cisplatin-DNA adducts). Riboflavin and folic acid being essential for DNA synthesis and repair, their supplementation perhaps protected the cells from damage by enhancing these mechanisms. That vitamin E, which showed maximum effect in VR induced IM cell apoptosis, could not decrease cisplatin-induced apoptosis, seems to corroborate the above observation, as it is mainly antioxidant in action. Although, vitamin E could prevent oxidative DNA damage, it may not help in repairing the already damaged DNA and thus it is clear that the pathway involved in cisplatin induced apoptosis is through DNA damage.

Mucosal damage is the common side-effect observed during chemo/radiotherapy of cancer and is the dose-limiting factor. Riboflavin and folic acid supplementation would help in preventing DNA damage, mutations and the occurrence of cancer as well as chemo-therapy related adverse effects. Vitamin supplementation (riboflavin, folic acid and vitamin E) especially as a mixture may be of help in preventing chemo/radiotherapy induced changes in IM cells by minimizing mucosal damage and supplementation through diet helps in maintaining intestinal function. Further studies are however needed to understand the detailed mechanism(s) involved in differential action of vitamins in different tissues.

2. TRANSMISSION ELECTRON MICROSCOPE (TEM) STUDIES ON DIET, PROTEIN AND VITAMINS RESTRICTED RATS

Investigators : L. Singotamu, B.Sesikeran, P.Udaykumar, S. Kalayanasundaram, A.Vijayalakshmi and P.Madhusudhana chary

Duration : 3 years

Date of completion : 2003

An experimental study has been initiated to see the possible cellular and subcellular components in which the alterations or impairments may occur in their Ultrastructure (due to nutritional deficiencies) Which include mitochondria, endoplasmic reticulam, golgi apparatus, lysosomes, microtubules, microfilaments, phospholipid bilayer, nucleolus, polysomes etc. A total number of 32 weanling rats were divided in to 4 groups of 8 each. The first group was fed on control diet ad libitum. The second group fed on 50% of diet consumed by first group i.e. control. The third groups were fed on 75% protein restricted diet. A fourth group fed on 50% vitamin restricted diet. Changes in body weight, plasma protein and Hb levels were monitored at periodic intervals. After 20 weeks of feeding, the rats were sacrified and perfused. Tissues like liver, kidney and intestine of rats were collected. Collected samples were washed in normal saline to remove excess blood and fixed in Karnovsky fixative overnight at 4°C and post fixed with 2% buffered osmium tetroxide for 2 hr at 4°C washed and dehydrated by passing through ascending grades of ethanol at room temperature and cleared in propylene oxide inflitrated with propylene oxide and resin mixtures. The samples were embedded with resin mixtures in the beam capsules and polymerized by keeping them in the vacuum oven for 24 hrs at 50°C and for another 24 hrs at 60°C. In previous studies where the experiment was conducted for 4 weeks and 8 weeks significant changes were not observed therefore this experiment was extended for 20 weeks. The prepared resin blocks were sent to EM Laboratory of All India Institute of Medical Sciences, New Delhi to carry out further studies. Ultrathin sections were cut with the help of Reichert ultracut microtome. Sections were collected on (Ultrathin sections 600°A to 900°A i.e. golden yellow to silver colored sections) double-coated 200-mesh copper grids, double stained with uranylacetate and leadcitrate. Sections were scanned by Philips 300 Transmission Electron Microscope. Required pictures were taken by 35-mm camera at appropriate magnifications. Negatives were developed and prints were made.

There were no major changes at cellular level, in the kidney, liver and intestine of experimental groups when compare to controls. However, cytosolic changes were seen in the form of cytoplasmic vacuolization in intestinal epithelial cells of 50% diet restricted rats. In 50% diet and vitamin restricted rats cytoplasm was disrupted in the

cuboidal epithelial cells that line the urinary tubules of kidney. There were no changes observed in the liver, except in 75% protein restricted rats where endoplasmic reticulum structure is disrupted. The protein synthesis is therefore hampered. Mean food intake during the twenty weeks experimental duration did not differ among protein and vitamins restricted groups rats compared to control, except diet restricted group where the food was reduced deliberately to 50%. In general, bodyweights matched with the food intake in control and vitamin restricted groups, while protein restricted and food restricted rats weighed significantly less (12% and 48% decrease respectively compared to controls). Weight gain (gm/gm diet) was lower in the animals on protein-restricted diet compared to other groups. Hemoglobin concentration reduced significantly in food and protein restricted rats while vitamin restricted rats showed no change compared to controls. On the other hand, serum protein concentration showed similar changes as hemoglobin in vitamin restricted rats whereas protein restricted and food restricted rats showed 12% and 8% decrease respectively compared to controls. No mortality is recorded in experimental groups. This may be due to adaptability and sustainability of biological system to varied conditions, even though there is a change in the diet intake pattern.

3. EFFECT OF COPPER AND MOLYBDENUM ON DEVELOPMENT OF SKELETAL FLUOROSIS IN RABBITS – HISTOPATHOLOGY STUDY

Investigators : A.L. Khandare, P. Uday Kumar and K.Venkiah

Duration : 1year

Date of completion : Oct. 2003

Endemic genu valgum affecting all strata of people particularly adolescents is a manifestation of chronic fluoride (Fl) toxicity. Epidemiological data shows that it is more common in areas where sorghum is a staple diet as compared to a rice based staple and sorghum grown in endemic areas has more molybdenum (Mo) than in other areas.

High Mo intake is associated with increase Copper (Cu) loss from bone as suggested by various studies and Cu is essential for maturation of collagen and bone. Since endemic genu valgum is more in adolescents, it is possible that deficiency of some nutrients during this period is responsible for genesis of genu valgum.

Presently, it is not known if the effect of Mo on Cu is exaggerated during high fluoride intake or whether high fluoride with Cu deficiency leads to genu valgum type of deformities.

AIM

To study the role of Cu and Mo in development of skeletal fluorosis in rabbits by undertaking haematological, histopathological and biochemical studies.

METHODOLOGY

A total of 30 rabbits, aged 2-3 months were divided into 5 groups of 6 each and fed on synthetic diet for a total period of 6 months. Group I was “control” and fed stock diet along with distilled water. Group II was “Fluoride group” while group III received fluoride and Mo. Group IV was fed on fluoride, Mo and Cu while group V had fluoride without Cu. Fluoride

was administered through water (150 ppm) while Cu and Mo (0.01% of diet and 0.1% of diet) were fed through diet.

At the end of 6 months, apart from twenty four hour urine and feces samples, blood samples were also collected and euthanized, during which all organs were collected, their weights and gross parameters recorded and subjected to routine histopathology sampling.

RESULTS

Histopathology study of various organs including heart, trachea, tongue, salivary glands, intestines, reproductive organs, muscle, spleen, adrenals and urinary bladder was unremarkable. Bone was not included for histopathology study (Table 16).

Table 16. Effect of Mo & Cu in fluoride toxicity - Histopathology study

Organs	Control	Fluoride	F+Mo	F-Cu	F+Mo+Cu
Kidney					
Normal	6/6 (0.0)	4/6 (66.7)	6/6 (0.0)	6/6 (0.0)	4/6 (66.7)
Tubulitis	--	2/6 (33.3)	--	--	2/6 (33.3)
Liver					
Normal	1/6 (16.7)	--	1/6 (16.7)	--	--
FAN	--	--	--	2/6 (33.3)	2/6 (33.3)
PVRCC	2/6 (33.3)	5/6 (83.3)	2/6 (33.3)	2/6 (33.3)	3/6 (50.0)
PPRCC	1/6 (16.7)	3/6 (50.0)	--	3/6 (50.0)	1/6 (16.7)
Fibrosis	3/6 (50.0)	4/6 (66.7)	4/6 (66.7)	5/6 (83.3)	4/6 (66.7)
Biliary hyperplasia	1/6 (16.7)	--	3/6 (50.0)	--	1/6 (16.7)
Brain					
Normal	3/6 (50.0)	2/6 (33.3)	5/6 (83.3)	5/6 (83.3)	1/6 (16.7)
Calcification	2/6 (33.3)	4/6 (66.7)	1/6 (16.7)	1/6 (16.7)	4/6 (66.7)
PVRCC	1/6 (16.7)	--	--	--	1/6 (16.7)
Spinal cord					
Normal	6/6 (0.0)	5/6 (83.3)	6/6 (0.0)	5/6 (83.3)	6/6 (0.0)
Calcification	--	1/6 (16.7)	--	1/6 (16.7)	--
Lungs					
Normal	--	--	--	1/5 (83.3)	--
PBRCC	5/6 (83.3)	2/6 (33.3)	2/6 (33.3)	1/5 (83.3)	1/5 (83.3)
CIPn Grade I	--	2/6 (33.3)	2/6 (33.3)	2/5 (40.0)	4/5 (80.0)
Grade II	1/6 (16.7)	1/6 (16.7)	2/6 (33.3)	--	--
Grade III	--	1/6 (16.7)	--	1/5 (83.3)	--

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* Values within the parenthesis indicate percentages.

FAN – Focal areas of necrosis
PVRCC – Peri-Vascular round cell collection
PPRCC – Peri-Portal round cell collection
PBRCC – Peri-Bronchial round cell collection
CIPn – Chronic interstitial pneumonitis

Nerve: Study of sciatic nerves was done both on H&E sections and a special stain (Kultschitsky's) was also used for assessing changes in numbers as well as myelination of nerve fibres. The total number of nerve fibres as well as the population of small & large fibre counts were done and analysed for changes, if any. It was seen that though there was a trend with increased large fibre population in Fl. treated and F+Mo+Cu groups as compared to controls, they were not statistically significant. Myelin stain did not show any differences in staining property between the groups.

Lungs: Showed changes, nonspecific in nature which are common to all colony bred animals.

Liver: Fibrosis was present along with mild inflammatory cell scattering within and with varying degree of biliary ductal hyperplasia. These could be attributed to the presence of parasitic infestation (coccidia) in rabbits while rest of the changes were nonspecific in nature.

Kidney: Peritubular inflammatory infiltrate in the medulla portion was seen only in the "Fl" and F+Mo+Cu group.

Brain: Presence of focal calcification and osteoid changes in the brain were observed in all groups including controls in the cortical areas of parietal lobes as well as ventricles (in perivascular areas).

Spinal cord: Two spinal cords of experimental groups (F and F-Cu groups) showed presence of focal calcification, mainly in the white mater peripherally. No other changes were seen.

CONCLUSION

It can be seen that the changes seen in various organs (including calcification in brain and spinal cord) were consistently present to varying degrees in the 'fluoride' group as compared to other groups. However, since many of the changes were simultaneously seen in the 'control' group also, it is difficult to ascertain their significance. It can also be noted that calcifications in the CNS which were not reported earlier, are being recorded for the first time in fluorotic cases which by itself is a unique observation. Their presence in the control group also, however cannot be explained, particularly with reference to calcific areas in the brain and fibrosis in the liver.

On statistical analysis, a trend could be observed with respect to above findings even though not significant. This is probably because of the small sample size and significant results may be obtained if the experiment could be conducted in a larger sample size.

VIII. EXTENSION AND TRAINING

1. PUBLICATIONS

The quarterly periodicals, namely, Nutrition (English), Poshan (Hindi), Poshana (Telugu) and a semi technical bulletin Nutrition News, covering popular articles of public interest and scientific information on nutrition were published. These periodicals have been well received by the public as is evident by their appreciating mails and also thirst for more information.

During the year, "Nutritive Value of Indian Foods", a popular publication has been reprinted with additional information on dietary fibre and essential fatty acids in common Indian foods. Twentyfive thousand copies of the publication have been reprinted, which include bulk order of 10,000 copies from M/s. Aristo Pharmaceuticals, Mumbai, for distribution among medical fraternity.

Other titles which have been reprinted on popular demand include "Dietary Guidelines for Indian – A Manual (English)", "Diet and Diabetes (English)", Menus for Low-Cost Nutritious Supplements (Suitable for North India), Low-Cost Nutrition Supplements for South India (Telugu) and Some Therapeutic Diets.

The publications of the Institute have been in great demand by the public and during this year **a total amount of Rs 7,59, 322/-** was generated through sales.

2. TRAINING PROGRAMMES

The four regular training programmes of the Institute viz. (i) MSc (Applied Nutrition) (ii) Post-Graduate Certificate Course in Nutrition (iii) Annual Training Course in Endocrinological Techniques and (iv) Techniques for Assessment of Nutritional Anaemias were conducted. This year a total of twenty eight candidates have attended these training courses and among them twenty two candidates were in-service candidates from various medical and home science colleges in different States.

In the training courses, care has been taken to expose the participants to the latest information both in theoretical as well as practical aspects in the field of nutritional sciences through lectures and demonstrations using multimedia educational approaches by the Institutes as well as expert guest faculty. Besides, visits to the Nutrition wards in the hospitals and community programmes were organized to expose the participants to prevailing nutritional problems in the population and IEC techniques in educating the community to prevent malnutrition.

In addition to the regular training courses an ad-hoc training programme was arranged for over 35 students doing Master's programme in various disciplines like Biotechnology, Microbiology, Biochemistry, Foods and Nutrition, Computers etc from different institutes in the country.

3. EXTENSION ACTIVITIES

3.1 Exhibitions

To commemorate 150th year of Indian Railways an exhibition was organized at Gooty, Anantpur district, on nutrition by displaying 35 posters on nutrition and health depicting functions of food, balanced diet and micronutrient deficiency disorders and dietary guidelines etc. on 9th and 10th April 2003

An exhibition was organized at Shilparamam during 1st to 7th November 2003 in connection with 1st Afro-Asian Games. Posters on health and nutrition were displayed and a sales counter of NIN publications was also put up (for sale of institutes publications) during the event. A huge turnout was there.

As part of the 91st session of Indian Science Congress, an exhibition and also a publication sales counter were organized in ICMR pavillion at Chandigarh during 3 – 7th January, 2004 in the Science and Technology exhibition on "Pride of India-Science Expo 2004.

A nutrition awareness camp and exhibition of posters on Nutrition specially developed for "Feeding Minds, Fighting Hunger" project were organised at Narsapur village, in association with Dangoria Charitable Trust on 7th Feb. 2004.

3.2. Popular Talks

- Delivered a series of extension lectures to the children and youth in the slums of old city of Hyderabad as part of the summer camps being organized by Confederation of voluntary Associations (COVA) during week ends in April and May 2003.
- Nutrition awareness camps were organized to the employees of Singareni Collieries Company Ltd. at Kothagudem and Bhupalapalli during April 21-22 and 2nd and 3rd May 2003 respectively. About 600 employees participated to the programme.
- A popular lecture on "Nutrition" was delivered to the primary teachers (50) at Kendriya Vidyalaya, Golkonda-2, on10th May 2003.
- A popular lecture on Health and Nutrition to the senior executives of State Bank of Hyderabad (160) at their Staff Training Center on 7th June 2003.
- An extension lecture on food and nutrition was delivered to the grade 3\6 to 9 Superintendents, Senior Superintendents, Foremen and inspectors during a training programme (70) on "health awareness " organized by Indian Airlines Limited on 27th June 2003.
- Scientists of ET Division served as resource persons and delivered lectures on Food and Nutrition projects – An Effective Way of Presentations in Science Fairs and National Children's Science Congress on 25th September 2003 to the science teachers (58) in an orientation programme organized by Andhra Pradesh Residential Educational Institutions Society, Hyderabad.
- A guest lecture on Nutrition/the food we eat was delivered through KU band (frequency) Ambedkar Open University to 1622 police personnel of the State police in 5 police training centres on 27th November 2003.
- A guest lecture on "Nutrition and Health" was given to the rehabilitation professionals (35) at Thakur Hariprasad Institute of Research and Rehabilitation for mentally handicapped on 18th December 2003.
- An inter-school competition in project work on "Iodine – quintessential micronutrient" has been organized in association with COVA and the participants displayed the project works in English and Telugu during February 7th and 8th 2004.
- Nutrition awareness programme was organized at science exhibition, organized by Dangoria Charitable Trust at Narsapur, Medak district during February 11th and 12th 2004.
- Organized nutrition awareness camps in 18 villages of Jangaon in association with World Vision, an international NGO during 8th to 19th March 2004. approximately 2000

villagers including women, adolescent girls participated in the programme. Extension lectures, posters on nutrition and health and preparation of low-cost nutritious recipes during the programme enthused the participants.

3.3. Radio talks

In connection with National Nutrition Week celebration popular talks on Balanced diet and Nutrition requirements for children of different age groups were broadcast in Telugu during the 1st week of September 2003 and were repeated several times during the year.

4. SPECIAL EVENTS

4.1. National Technology Day

A popular lecture aided by posters on nutrition was delivered to adolescent children in the schools of old city in association with COVA on May 11, 2003.

4.2. National Nutrition Week

- A nutrition awareness camp on "Health and Nutrition" and followed by well baby contest were organized at Domaragunta village, Murugu Mandal, Medak district on 7th September 2003 in association with an NGO Society for Rural health, Hyderabad and Food and Nutrition Board, Hyderabad.
- Integrated Nutrition Education Camp was organized at Lady Doak College, Madurai, Tamil Nadu.
- Inter-school elocution competitions were organized in Urdu and Hindi on this year's theme – 'Nutrition and National Development'. An Inter school Nutrition quiz contest was also organized at the institute.

4.3. World Food Day

- Nutritional awareness programmes were conducted for about 400 employees and their families of Andhra Pradesh Power Generation Corporation at Vijjeswaram and Mothugudem, Vishakapatnam District, between October 14 and 16, 2003.
- A performance of children's play covering aspects like hunger, nutrition and food security developed as part of Feeding Minds, Fighting Hunger project was organized for the benefit of several schools in the old city of Hyderabad.

4.4. National Science Day

Two extension lectures were organised for the school children of 8th, 9th and 10th classes of Atomic Energy High School – II, Hyderabad, on 26th February 2004. 150 students participated in the programme.

4.5. International Women's day

A nutrition awareness camp was organized at Chintapally and China bail villages for women on 8th March 2004 in association with World Vision Hyderabad on 8th March 2004. About 180 women participated in the programme.

4.6. Inauguration of NIN's website

Sri. JVR. Prasada Rao, IAS, Secretary, Health and Family Welfare, Government of India, and Prof. N.K. Ganguly, DG, ICMR, were at the institute on 17th March 2004 for **deliberations on the report "Iodine Deficiency Disorders"** brought out by the Division of Field Studies. Sri.Prasada Rao also inaugurated the Institute's website, www.ninindia.org

5. WORKSHOPS CONDUCTED

As a part of orienting all the prospective guides in NTR University of Health Sciences on Research Methodology, two day interactive programmes were organized twice (9-10, July, 2003 and 24-25 Feb. 2004) in two batches.

A teachers' training workshop was conducted for the biological science teachers as a part of the project titled "Implementation of Feeding Minds, Fighting Hunger Programme – An Exploratory Study" on 18th July, 2003.

A One-day Orientation Workshop on Nutrition for school teachers was conducted for teachers in association with Confederation of Voluntary Associations (COVA) on 6th January 2004.

6. PUBLIC AND MEDIA RELATIONS

The Nutrition Museum continues to attract students from school and colleges, health workers, nurses and NGO groups from all parts of the country. Lecture-cum-video programmes on various nutrition themes were conducted for these visitors in batches. A total number of 86 groups consisting of 2580 students from ten States (Andhra Pradesh, Gujarat, Maharashttra, Kerala, Tamilnadu, west Bengal, Madhya Pradesh, Uttar Pradesh, New Delhi and Punjab) visited the institute during the year.

Technical information was provided to general public on nutrition and health-related aspects and dietary counselling was offered to the needy general public.

Reporters from several newspapers interacted with the scientists of the Institute working on different aspects published research highlights. In addition, articles from the Institute's periodicals were also picked up by various newspapers in different Indian languages. All the extension activities of the institute were covered well by the local media in English, Telugu and Urdu.

The staff of the Extension and Training Division took active part in ensuring media coverage for all the special events organized by the institute. Curtain raisers, press releases and follow-up reports were coordinated by the staff. Staff was also actively interacting with media and was successful in improving visibility of the institute as well as ICMR.

ETV – Urdu (TV channel) in coordination with Extension division made a television capsule on NIN's training programmes as a part of its career guidance programme.

Expertise of NIN's scientists was also used by the Educational Media Research Centre (EMRC), Hyderabad in making educational films on nutrition and food security .

The staff of ET division assisted in the designing, development and editing of brochures and attractive draft proposal for the proposed National Animal Resource Facility near Hyderabad.

A set of seven educational folders on various aspects of nutrition for educating the NSS volunteers was developed as part of "Development of communication strategies to improve nutrition and health related knowledge of NSS volunteers" - project.

7. DEVELOPMENT OF NIN'S WEBSITE

ET division took active role in evolving design, creating and launching of the institute's website, www.ninindia.org. The staff of the division were responsible for coordination, assimilation and editing the content, working as interface between various divisions of the institute and the firm that developed the website.

Various other related activities like photography, designing of images (Photoshop) were also done by the staff of division. Regular updations and additions to the content of NIN website are also being carried out.

8. DEVELOPMENT OF PORTABLE EXHIBITION

A state-of-the-art portable exhibition consisting of collapsible structure, two roll-ups and two compacts with built in lighting facility was developed. The portable exhibition in English, with messages on dietary guidelines for Indians, nutrition deficiency disorders and history & evolution of the institute is expected to serve as an information aid to the community. It is proposed to develop similar portable exhibition in various Indian languages to start with in Telugu and Hindi as the one in English is very popular.

9. REVAMPING OF THE EXISTING MUSEUM IS BEING ATTEMPTED

LIST OF PARTICIPANTS FOR THE YEAR 2003-2004

MSc (APPLIED NUTRITION) COURSE (16th June 2003 - 12th March 2004)

1. Lt. Col. Rajat Prakash, MD (SPM)
   Dy. Asst. Director Health, HQ Punjab, Haryana & Himachal Pradesh (1),
   Sub Area, Ambala Cantt., Haryana

2. Dr.Kailash Bhattacharjya, MD (Biochemistry)
   Asst. Professor, Dept. of Biochemistry, Assam Medical College, Dibrugarh, Assam

3. Dr.Jasleen Virik, MBBS
   Medical Officer, PCMS-1, Directorate of Medical & Family Welfare,
   Sector-34, Chandigarh, Punjab

4. Dr.Nitin Sitaram Gajre, MBBS, PG Dip. Nutr. & Dietetics
   242/9485, 3rd Floor, Kannamwar Nagar-2
   Vikhroli (E), Mumbai – 400 083, Maharashtra

5. Dr. Sajnikar Sanjay Jagdish, MBBS
   19/1, Avanti Society, Indrayani Nagar, Sector-2, Bhosari,
   Guru Nanak, Pune – 411 026, Maharashtra

6. Mr. Prashant Tripathi, MSc (Biochemistry)
   P-5, Medical College Campus, Kanpur – 208 002, Uttar Pradesh

7. Ms. K.Valanthina Kuraizi, MSc
   (Food Service Management and Dietetics)
   3/47, Ambalakar St. Parthibanur – 623 606, Ramnand Dt., Tamil Nadu

PG CERTIFICATE COURSE IN NUTRITION (5th January to 12th March 2004)

1. Dr.Surya Prasad Pannala, MBBS
   Civil Asst. Surgeon & Inservice PG Diploma in Public Health
   Department of SPM, Gandhi Medical College, Secunderabad

2. Mrs. P.K.Vageeswari, MSc (AN)
   233, Sector A, AWHO, Gunrock, Secunderabad – 500 009

3. Ms. Vandana Luthra, MSc (AN)
   C/o Sh. DR Luthra, H.No. 942, Sector 13, Urban Estate
   Kamal, Haryana – 132 001

4. Ms. Kavitha Chandrasekhara Menon, MSc (AN)
   Krishna Kripa, Ambalapuram, West Yakkara, Palakkad – 678 001

5. Dr.Vikram Anant Rajadnya, MD (Pharmacology)
   C/o AA Potdar, 250, A, Ward Shivaji Peth, Kolhapur – 416 012

6. Daphna Dror
   318 A Parnassus Ave, San Franscisco CA, 94117, USA

ANNUAL TRAINING COURSE ON ENDOCRINOLOGICAL TECHNIQUES AND THEIR APPLICATION (18th August - 30th September 2003)

1. Dr.Sepuri Madhuri, MD (Bioch.)
   Asst. Professor, Department of Biochemistry
   Andhra Medical College, Visakhapatnam

2. Dr. Savitri Rajanna Sahukar, MD (Bio.)
   Lecturer, Dept. of Biochemistry
   JJM Medical College, Davangere

3. Mr. Pradeep Kumar KM, MSc (Bioc.)
   Scientific Assistant, Dept. of Biochemistry
   Medical College Calicut, Calicut Medical College, Kerala

4. Dr. Suchanda Sahu, MD (Bioch.)
   Lecturer, Dept. of Biochemsitry, Christian Medical College, Ludhiana

5. Dr. Vani Gupta, MD (Physiology), DNB
   Lecturer, Lucknow University, Lucknow

6. Mr. Surapaneni Sivaprasada Rao, MSc (Analytical Chemistry)
   Ratna Laboratory, No.73-1-12/A, Opp. Durga Mahal
   M.G.Road, Patamata, Vijayawada – 520 010

TECHNIQUES FOR ASSESSMENT OF NUTRITIONAL ANAEMIAS (15-26th March 2004)

1. Dr. R. Nagamani, MD (Gen.Med)
   Asst. Prof. of Medicine, Gandhi Medical College, Hyderabad

2. Dr. M.N.Shyam Sunder, MD (Gen. Med.)
   Asst. Prof. of Medicine, Gandhi Medical College, Hyderabad

3. Dr. Byram Bheeshma, MD (Pathology)
   Associate Professor, Dept. of Pathology
   Kakatiya Medical College, Warangal

4. Dr. Arunima Banerji, MD (Pathology)
   Asst. Prof, Surat Municipal Institute of Med. Education & Res., Surat

5. Dr. Mohan Kondiba Doibale, MD (SPM)
   Assoc. Prof., Dept. of SPM, Govt. Medical College,
   Vazirabad, Nanded

6. Dr. (Mrs.) V.Raji Sugumar, MSc (Home Science), M.Phil, PhD, PG Diploma
   Lecturer in Home Science, Bharathidasan Govt. College, Pondicherry

7. Dr.T.K.Shaanthy Gunasingh, MD (O & G)
   Asst. Prof., Dept of O & G, Govt. Kilpauk Medical College, Chennai

8. Dr. A. Anandhi, MD (O & G)
   Asst. Prof. Dept. of O & G, Govt. Kilpauk Medical College, Chennai

9. Dr. Reena Vijay Wagh, MD (Biochem.)
   Lecturer, Dept. of Biochemistry, Govt. Medical College, Nagpur

VI. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE (FDTRC)

A. FOOD SAFETY

1. FLUOROSIS IN YOUNG CHILDREN IN VILLAGE OF BIHAR STATE

Investigators : Arjun L. Khandare, R. Hari kumar, B. Siva kumar

Duration : 6 months

Date of completion : December 2003

The study was conducted in response to lay press reports of occurence of new forms of bone deformities among young children in "Kachariadih" village, of Nawada district in Bihar state of North India, where fluoride levels in drinking water were reported to be high.

The objective was to establish the diagnosis of the new form of skeletal deformities, and find out the aetio-pathogenesis of the bone deformities and then to suggest possible remedial measures for improving the situation.

METHODS

Information provided on one hundred and twenty villages by the State Public Health Engineering Department in the district revealed that the fluoride levels in the sources of drinking water ranged from 0.8 to 3 ppm, and the survey in the reported village "Kachariadih (2km), and four other villages surrounding the reservoir, namely P.Hardia (2km), Bhaur (0.5km), Chitrakauli (6km) and Chamutha (18km), indicated that the skeletal deformities were prevalent only in one village namely Kachariadih and its hamlet, P. Hardia, where the fluoride levels ranged from 3.5-14.5ppm and 1.4 - 6.2ppm respectively. Later a case control study was carried out in the high fluoride village and a control village with normal fluoride levels. The study included clinical examination, random diet survey, collection of drinking water and urine and blood samples for biochemical investigations.

The case control study was undertaken by comparing the data obtained from affected village, " Kachariadih" having 54 HHs and about 240 population and a non- affected control village, "Chitrakauli" with 197 HHs and about 1443 population, located 5 km towards south of affected village. In order to compare the data, 1/3 of the HHs from the control village were selected randomly while all the HHs of affected village were considered.

Water samples were collected from all drinking water sources of affected village and from 30% of sources from the non-affected village. Spot urine samples were collected from a sub sample of the children covered for the clinical examination. Purposive blood sample collection was done on children with deformities affected high fluoride (AHF, n=14), children without deformities unaffected high fluoride (UAHF,n=14) from high fluoride village and the children low fluoride (LF,n=7) from low fluoride village. The blood samples were brought under cold conditions to a Base Center, where the serum was separated and were brought to National Institute of Nutrition, under cold conditions (4^{o}C) for biochemical analysis.

CLINICAL EXAMINATION

Clinical examination for dental mottling, skeletal deformities (genu varum, genu valgum, anterio posterior bowing of tibia, Kyphosis, scoliosis, exostosis, epiphysial

enlargement etc), muscular tenderness, neck rigidity, stiffness of joints and mental retardation, was conducted on all the subjects in the affected village and on subjects from about 1/3 of the HHs in the control village.

Radiological examination

X-Rays of the left tibia & fibula (lateral and anterior-posterior views) were taken on a sub sample of affected children (15 out of 48) along with a clinically normal child.

Biochemical Analysis

Fluoride content of water and urine samples and, heavy metals like lead, cadmium, silicon and molybdenum in water were estimated. Other minerals like calcium, magnesium, zinc and copper were estimated in water, urine and serum. Alkaline phosphatase activity in serum, creatinine, phosphorus in serum and urine and urinary proteins were analyzed by kits supplied by "Rosche". **Urinary protein was analyzed by strip method.**

Food and Nutrient intake

Diet survey was carried out by semi quantitative food frequency questionnaire method, on 40 HHs in each of the High fluoride (AHF) and low fluoride control (LF) villages, for calculating calcium and phosphorus intakes. The dietary intakes of different nutrients were calculated per Consumption Unit using food composition tables.

RESULTS

i. General observations

A majority of the population in the affected village belonged to Scheduled Castes (81%) and backward classes, while in the control village the proportion of Scheduled caste population was 32%.

The only sources of drinking water in both villages were bore wells of >80 feet depth. The study also revealed that surface water from the reservoir invariably contained several times less fluoride (0.9 ppm) than the water from the bore wells in the HFV (mean of 7.9 ppm). It was observed that the population in both the villages was adequately exposed to sunlight.

ii. Clinical manifestations

Clinical manifestations such as dental mottling of various degrees and skeletal deformities of different types are prevalent in the affected village. Dental mottling was not noticed in 1-5 year age group in both villages and genu valgum was as high as 20.5% in 1-5 year children of affected village, followed by antero posterior bowing of tibia and fibula either exclusive or associated with genu valgum or genu varum (13.6%) and genu varum (2.3%).

In the age group of 6-11 years children, the prevalence of dental mottling was to the extent of 84%, while that of skeletal deformities, genu valgum, antero posterior bowing of tibia and fibula and genu varum was 14, 17and 8% respectively.

Among the adolescents (11-18 yrs), dental mottling was observed in 92%, skeletal deformities like anterior bowing was prevalent among 12% followed by genu valgum, genu varum in 4% each of the subjects respectively.

While no skeletal deformities were observed in the control village, dental mottling was observed in only 6% of the population.

Pain in lower limb commenced as the first sign once the child was weaned off from breast and started eating food and drinking water. According to the residents, the painful, crippling deformities in children developed as soon as child started to walk.

It was also observed that in severe cases, the deformity prevented the subjects from assuming postures and performing movements of their choice, thereby crippling them.

Age wise distribution of bone deformities among the affected children showed that it was uniformly high. About 20% of bone deformities (genu valgum) occurred in early age up to 9 years and more predominant in 1-5 years old children. On the other hand, anterior bowing and genu varum showed maximum prevalence in 6-9 years and was lower on both sides of this age group (below 6 years and above 9 years). Anterior bowing follows next (<20%) in magnitude after genu valgum. No perceivable differences were observed among males and females.

iii. Radiological findings

'U' shaped deformity of both tibia and fibula, thickening of cortex was seen in the X-ray of a male subject aged 12 years. Similar 'U' shaped deformitiy of both bones of legs with multiple stress lines and osteoporosis of both the bones were observed in X-ray in another male subject aged 14 years. Expansion of bones with fraying of epiphyseal ends of tibia and fibulal with mild periosteal reaction are seen in the X-ray of a male subject of 5 years. Extensive ligamental calcification, bony trabeculations were also seen in older children. Rachitic changes were confirmed by radiologist.

iv. Bio-chemical findings

Mean fluoride content in drinking water sources in affected village (7.9±4.15 ppm) was significantly higher than that in control village (0.6±0.31 ppm) There was no difference in calcium, magnesium, zinc, copper, lead, cadmium, silicon and molybdenum content in drinking water samples collected from control and affected villages.

As the mean ages of children were not different amongst the three groups, the biochemical findings of the affected children from high fluoride village (AHF) were compared with those without bone deformities (UAHF) and also with controls from low fluoride (LF) village. Mean urinary fluoride levels of children of both AHF and UAHF groups were significantly higher than those of children from LF. Serum alkaline phosphatase activity among the children of AHF group was significantly higher than those of UAHF group in the same as well as those of LF group. Mean serum vitamin D (25 OH - D_3) was significantly lower in the AHF and UAHF groups as compared to those of LF group. There was no significant difference in serum vitamin D (25 OH - D_3) among the children of AHF and UAHF groups from the same village. However, it (vit. D) was on the higher side in the children belonging to UAHF group which was not significantly different. Serum IPTH was also significantly higher in the children of AHF and UAHF as compared to the children of LF group from control village. Serum calcium levels in AHF (with bone deformities) were significantly lower than those of UAHF (without bone deformities) from the same village as well as those belonging to LF village. The serum phosphorus levels in the children of high fluoride village (without bone deformities) were significantly higher than those of children with bone deformities from high fluoride village as well as children from LF village. There was proteinuria and Phosphaturia among

the children of both the villages. There was no significant difference of serum phosphorus levels in the children with bone deformities and those of children from LF control village. There was no significant difference in serum creatinine, Zn, Cu, Mg as well as urinary Ca, Zn, Cu and Mg contents between the affected and control villages.

v. Food and nutrient intake

It was observed that the population in the HF village has been sustaining on a cereal diet (590g/cu), with significant quantities of potatoes (187g/cu), very low milk intakes and fewer varieties of vegetables, whereas in the LF control village the consumption of vegetables, milk, fats & oils and sugar has been comparatively better

The mean intake of calcium in the AHF group was significantly lower (352 mg, $p<0.05$), while the intake of phosphorus (1887 mg) was significantly higher ($p<0.05$) as compared to LF control village.

CONCLUSIONS

Very young children suffered from these deformities with radiological changes, while the adults manifested only stiffness of the spine which strongly suggests that, in addition to high levels of ingestion of fluoride, some recent environmental changes could have contributed to the genesis of the syndrome. It can be concluded that the clinical and radiological changes (rachitic) of bones in young children, associated with high serum alkaline phosphatase, low serum Vitamin D, calcium, and proteinuria associated with high fluoride in drinking water may be due to secondary vitamin D deficiency.

2. ROLE OF FOOD PROCESSING ON PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND DEVELOPMENT OF RECIPES WITH HIGH ANTIOXIDANT ACTIVITY

Investigators : Rita Saxena, K.Venkaiah and M.Raghunath

Duration : 5 years

Date of completion :

Free radicals, generated during the course of normal metabolism, are neutralized by the endogenous antioxidant system. However, external environmental conditions like stress, chemical carcinogens, irradiation and smoking etc generate more free radicals, which the endogenous antioxidant system may not always be able to cope up with neutralization.

Plant foods are good sources of antioxidants and phenolic compounds (PC) are potent antioxidant substances ubiquitous in plant foods. This study is an attempt to generate the data base on the phenolic content and antioxidant activity (AOA) of plant foods commonly consumed by the Indian population. For this purpose three samples of each plant food belonging to various food groups (most frequently consumed by the society) were purchased from three different markets located in three distant geographical parts of the twin cities (Mehdipatnam, Uppal and Monda market , Secunderabad). They were pooled and analysed as one sample from each market for their phenolic content and antioxidant activities by the methods described earlier.

RESULTS

The salient observations of the study are summarized below:

AOA was observed to be the highest in black pepper and the lowest in sunflower oil. The AOA of Redgram dhal, whole blackgram, blackgram dhal and ginger followed that of black pepper in decreasing order. The AOA of other foods analysed was of intermediate intensity.

Phenolic content of black pepper was the highest and ground nut oil and sunflower oil had no phenolic substances in them. The phenolic content of other foods tested ranged in between them. Among the foods with high AOA ginger had high phenolic content, while redgram dhal, whole blackgram and blackgram dhal had low phenolic content.

In line with discrepancies observed in the phenolic content of foods and their AOA , there was no correlation between the two parameters in most of the foods except whole blackgram, spinach, onion, black pepper in which the two parameters showed positive correlation . These results suggest that PC may be an important contributor to AOA of these foods but not in others. Keeping in view that plant foods contain a variety of oxidants / antioxidants in which phenolics are only one class of compounds, the results although puzzling do not seem to be altogether unexpected (Table 17).

Table 17. Antioxidant Activity* and Phenolic Content** of commonly consumed raw foods

Food stuff	AOA content (Mean ± S.D.)	Phenolic content (Mean ± S.D.)
Rice	8.3 ± 3.3	12.1 ± 8.2
Wheat	7.6 ± 2.1	22.2 ± 4.0
Black gram dal	1.1 ± 0.2	26.1 ± 0.7
Black gram (whole)	1.0 ± 0.1	59.6 ± 6.8
Red gram dal	0.9 ± 0.4	25.3 ± 7.3
Amaranth leaves	3.1 ± 0.3	84.9 ± 13.0
Spinach leaves	2.8 ± 1.2	33.8 ± 7.3
Bitter gourd	4.9 ± 0.6	53.9 ± 12.0
Tomato	2.3 ± 0.6	36.1 ± 2.9
Carrot	5.8 ± 2.9	9.0 ± 0.7
Onion	3.2 ± 1.1	39.2 ± 6.1
Black pepper	0.4 ± 0.1	190.8 ± 41.9
Garlic	4.8 ± 0.6	44.3 ± 12.7
Ginger	1.2 ± 0.5	111.3 ± 8.2
Groundnut	2.3 ± 0.9	93.3 ± 13.6
Sesame	3.7 ± 0.3	89.1 ± 14.4
Groundnut oil	12.1 ± 5.1	0.0 ± 0.0
Sunflower oil	18.4 ± 9.3	0.0 ± 0.0

* Expressed as mg of food required for 50% Inhibition of oxidation of LA and β-Carotene

** Expressed as mg of gallic acid equivalent in 100g of raw food stuff.

3. DIETARY EXPOSURE ASSESSMENT OF FOOD ADULTERANTS AND CONTAMINANTS

Investigators : Ramesh V. Bhat, V. Sudershan Rao and S. Babu

Duration : 1 year

Date of completion :

BRIEF OUTLINE

There have been numerous outbreaks of foodborne diseases in India due to ingestion of contaminated food. The consumption of foods contaminated with various environmental contaminants, toxicants and adulterants could lead to deleterious effects in humans depending on the level of contaminants/toxicants/adulterants. There is a need to assess contaminats/ toxicants/adulterants in foods.

OBJECTIVES

The present investigation is aimed at exposure assessment of food contaminants and adulterants under normal circumstances in vulnerable state like Orissa.

Diet survey results of winter season and laboratory analysis of food samples for adulterants and contaminants was reported in last annual report (2002-2003). Diet survey in the same household both in rainy season and summer season were carried out in the current year.

The average energy intake was 1975.58 ± 1127.41 calories in summer and 1276.4 ± 565.7 in rainy season. The food sample i.e. rice, maize, redgram dhal, bengal gram flour and edible oils were analysed for organo chlorine pesticides DDT, BHC and endosulfan. Only edible oils 9 out of 19 contained endosulfan residues ranging from 46.08 to 197.8 ppb.

Analysis of total diet from the households of Panasguda and Bilmala indicated that both endosulfan and DDT were well below their corresponding Acceptable Daily Intake i.e. 0.02 mg/kg bw. And 0.006 mg/kg bw. Similarly heavy metals like arsenic, cadmium, lead mercury were also less than their corresponding Acceptable Daily Intakes.

The study indicates that there is wide variation in energy intakes in different seasons. Food consumed by the tribal people are free from adulterant i.e. argenome and *lathyrus sativus*. Tribals are exposed to dietary contaminants like pesticide residues and heavy metals. However risk assessment shows that their intake is below the Acceptable Daily Intake. The mycotoxins were present in maize but were absent in mango kernels they consumed.

4. INVESTIGATIONS IN THE OUTBREAK OF SUSPECTED EPIDEMIC DROPSY IN MADHYA PRADESH

Investigators : Ramesh V. Bhat and S.Babu

Duration : 3 months

Date of completion : Sept. 2003

BACKGROUND

An outbreak of suspected epidemic dropsy occurred in two towns of Madhya Pradesh i.e. Shivapuri and Sheopur during July 2003 and the outbreak was investigated at the request of Ministry of Health and Family Welfare.

OBJECTIVE

To find out etiological agent causing the outbreak.

WORK DONE

The team consisting of scientists from Ram Manohar Lohia Hospital, New Delhi, National Institute of Nutrition, Hyderabad and National Institute of Communicable Diseases, Delhi visited Gwalior on 6th July 2003 and with the help of sate officials visited Shivpuri and Sheopur where large number of patients were admitted for treatment.

A total of 480 people were affected. Most of the cases admitted in the hospital were in the age group of 15-40. However, according to the doctors all age groups except infants visited OPD. All the patients belonged to low socio economic strata. Most of the initial cases were from areas nearer to township of Shivpuri such as Neelghar Chauraha, Fatehpur, Old Shivpuri, Jhansi Tiraya, Barodi. Later cases came from villages under PHCs of Pohari, Satanwara, Kolaras, Narwar, Manpura and Badar. Multiple cases had been reported from the same family for example in a family of 18 persons, 17 had similar complaints simultaneously. Except, head of the family who was away from home was spared. In a group of 25 road construction workers, who were staying in make shift accommodation at Pohari-Shivpur road became ill after eating in a common mess, all had illness. All cases gave history of consuming mustard oil purchased (unbranded loose oil) from local sources. No mortality has been reported.

1. Foods of all affected families have been prepared in mustard oil, wheat and red gram dal.
2. All 14 oil samples collected from the affected households of Shivpuri and Sheopur showed the presence of argemone oil by TLC/HPLC.
3. The clinical features and epidemiological data suggest an outbreak of epidemic dropsy in towns of Sheopur and Shivpuri and its neighborhood.

5. REPORT ON THE FIELD TRIP TO PLACHIMADA IN PALAKKAD DISTRICT IN KERALA

Investigators : S.Bapurao, V.Sudershan Rao, G.J.J.Babu and S.A.Brinda

Duration :

Date of completion :

BACKGROUND

There were news reports in the middle of August 2003 implicating the factory causing heavy metal pollution through distribution of the waste sludge to farmers as manure. The factory, Hindustan Coca-Cola Beverages Pvt. Ltd. (HCC BPL) was established in 1999 in Plachimada Village of Perimatti Panchayat of Palakkad District. The management has been importing the concentrate and getting it diluted before bottling the soft drinks. The analysis by Kerala Pollution Control Board (PCB) also showed very high levels of cadmium,

and lead in the sludge samples. It was hypothesised that the cadmium content of paddy (Rice) would increase when grown on sludges containing high levels of cadmium resulting in disease, similar to itai-itai, which was known to occur due to cadmium toxicity. Therefore the study was undertaken with the following objectives and the results of which are reported here.

i) To collect the sludge dumped/distributed by HCCBPL.
ii) To collect food samples preferably paddy grown on lands enriched with sludge.
iii) To survey for any possible adverse affects, on the health of the people.

The team members met the District Collector and District Medical Officer and requested for help. They readily agreed and extended full co-operation in the study.

MATERIALS AND METHODS

Plachimada and other adjoining villages in Kerala State are spread over. The factory is located very close to Plachimada Village in Perimatti Panchayat. To the north of Perimatti panchayat is Nallepalli panchayat. The sludge from the factory was supposed to have been dumped and distributed initially free as manure to a few farmers in a few villages in these panchayats by two contractors. Observing this many more farmers asked for additional sludge, which was supplied. Contrary to news paper reports that it is used in rice fields, we have observed that the sludge was used only for areca nut and coconut trees but not for paddy fields, as it (sludge) contained glass pieces.

MATERIALS

a) *Sludge*: It was not possible to collect the sludge samples from the factory. Therefore samples of sludges were collected from farms wherever available. It is possible that sludge samples could have been contained a little bit of soil while collecting. A total of 8 samples of sludge were collected. Sludge no.1, was given to us by the Medical Officer, Nanniode Panchayat while other sample were from fields. The medical Officer had collected the sample and wanted to get analysed by someone who had the facilities. As the samples of sludges did not appear homogenous, the duplicate analysis are given as individual values and not as average.

b) *Samples of water:* A total of II samples of water from bore wells in and around the factory were collected for heavy metal analysis.

c) *Vegetables*: Five samples of vegetables from different fields, along with two one sample of arecanut and two fresh coconuts, grown on sludges by farmers were also collected for analysis.

d) *Paddy samples:* After an extensive survey of villages, we could get only two samples of paddy. The persons who gave these samples said that they were not grown on the sludges. In fact many farmers were of the opinion that these samples of sludges have glass pieces and therefore cannot be used in paddy fields as it will hurt people planting the saplings and harvesting.

e) Clinical examination of children and adults living in close vicinity of the factory was carried out and eight random urine samples were collected from residents who were examined.

METHODS

Five to six grams of sludge or vegetables or samples of arecanut were digested using HNO_3 and wet digestion procedure and made to 25 ml while water samples were aspirated into plasma as such. Heavy metals were measured with Direct Current Plasma Atomic Emission Spectrometer (DCPAES). In DCPAES plasma is created by ionizing the Argon gas between one cathode and two anodes in the form of "Y" shape. Lead (Pb), Cadmium (Cd) and Arsenic (As) were measured in all the samples with appropriate emission lines. (Pb: 368.3, Cd : 225.8, As : 193.7 nm). Suitable reagent blanks were prepared for correction of other emissions. Each value is an arithmetic average of 10 readings with co-efficient of variation 3 to 5%.

RESULTS

1. *Sludge samples:* Except two samples, the other six samples of sludge were very low with respect to Pb and Cd content (Tabel-18). The sludge samples given by the Medical Officer of Nanniode and the one collected from Sreedhar's farm II showed high values for both Pb and Cd. However, lead (Pb) values were higher and cadmium (Cd) values were lower than those reported by the Kerala Pollution control Board (KPCB).
2. *Vegetables:* The Pb and Cd content are given in Table 19 and 20. All the values are within the range observed for the vegetables.
3. *Water samples:* Pb and Cd content of water samples are given in Table-21. The Pb values are relatively high in all the samples and Cd values are within the levels reported in the literature. Although the Pb values in water samples were higher than the normal drinking water standards, these values for Pb were common in bore well waters.
4. Arsenic content was less than detection limits in all the samples collected.
5. *Urine samples:* Urine samples from children were collected, examined and the results are given in Table-22. Protein content of all urine samples were within normal range except one was above normal range.

Table 18. Sludge samples from farming in Palakkad

Sl.No	Place of collection	Lead µg/g	Cadmium µg/g
Sl	**PHC Nannoide**	434	6.6
S1D	**(Medical Officer)**	309	9.5
S2	**Sreedhar's Farm - I**	3.71	0.236
S2 D		5.05	0.015
S3	**Sreedhar's farm - II**	595	5.6
S3D		522	7.5
S4	**White Precipitated after**	< dl	0.16
S4D	**bottle wash**	<dl	0.003
S5	**Chintamara**	3.0	0.003
S5D		2.1	0.005
S6	**Chintamara**	0.63	0.004
S6D		<dl	0.009
S7	**Chintamara**	1.4	<dl
S7D		1.08	0.029
S8	**Chintamara**	1.19	<dl
S8D		1.48	<dl

"D" shows duplicate.
<dl means less than detection level.

Table 19. Vegetables from Palakkad

Name of sample	Lead µg/g	Cadmium µg/g
Lady's finger -1	2.83	0.042
Lady's finger-2	0.641	0.345
Horse gram-1	0.34	0.01
Horse gram-2	0.638	0.044
Raw banana-1	<dl	<dl
Raw banana-2	<dl	0.019
Lemon with rind-1	3.07	0.011
Lemon with rind-2	0.464	0.016
Lemon inner pulp-1	0.559	0.014
Lemon inner pulp-2	0.578	<dl
Paddy (New)-1	<dl	0.055
Paddy (New)-2	<dl	0.032
Paddy (Old)-1	<dl	0.033
Paddy (Old)-2	<dl	<dl

<dl means less than detection level.

PFA limit Lead 2.5 µg/g
Cadmium 1.5 µg/g

Table 20. Nut samples of plants grown in sludge from Palakkad

Name of Sample	Lead µg/g	Cadmium µg/g
Areca nut (fresh)-1	0.612	0.045
Areca nut (fresh)-2	0.244	0.013
Coconut -1	0.023	<dl
Coconut -2	<dl	0.017
Coconut water-1	<dl	0.002
Coconut water -2	<dl	<dl

<dl means less than detection level.

Under PFA the above items come under foods not specified :
Lead 2.5µg/g
Cadmium 1.5µg/g

Table 21. Water samples from open wells and borwells Palakkad

Sl.No	Place of sampling	Lead µg/ml	Cadmium µg/ml
21	**PHC perumatti**	0.33	0.005
22	**PHC perumatti**	0.26	<dl
23	**PHC 1**	0.52	0.023
24	**PHC 2**	0.41	<dl
25	**No. 2 palakkad**	0.25	0.001
26	**Open well 2**	0.22	<dl
27	**Borewell 2**	0.48	0.01
28	**Openwell 1**	0.49	0.008
29	**Borewell 1**	0.38	0.015
30	**Borewell 4**	0.36	<dl
31	**Borewell 6**	0.15	0.023

<dl means less than detection level.

EPA standards for water : Lead 0.015 µg/ml
Cadmium 0.005µg/ml

Table 22. Urine Analysis - Report of Kerala

Sl.No	Name	Urinary Total Protein mg/dl
1	**Subash - M/38**	1.62
2	**Bijue - M/44**	3.70
3	**Laxmi - F/15**	11.29
4	**Vasanthi - F/29**	2.55
5	**Dyawani - F/30**	19.90
6	**Shelvi - F/24**	5.25
7	**Shashikala - F/27**	11.906
8	**Rani - F/27**	7.76

Urinary total protein - 1 - 14 mg/dl
(Reference range clinical chemistry - Tietz)

DISCUSSION

All the samples had very low cadmium content not favouring the hypothesis that "Cd toxicity is rampant". Further, paddy seems to have not been grown on the sludge supplied to them for reasons best known to the farmers. Since the area was covered extensively over 3 days, the possibility did not exist of paddy growing on the sludge, as seen by the values for Pb and Cd content of paddy samples analysed.

As far as Pb is concerned, water samples and two sludge samples had high content of lead compared to the levels prescribed for drinking water. However the values are not alarmingly high. The source for these metals in the samples could not be ascertained.

Kerala Pollution Control Board recently published their analytical results for Pb and Cd in sludge samples which were higher than the values reported here (Times of India).

As per general norms, the sludge was expected to be used for the landfill. With in a few days of the news report, it is understood that factory has lifted or removed all the sludge dumped at sites in different villages and at the time of our visit no sludge could be seen in that area.

CONCLUSIONS

1 As was apprehended, the paddy was not grown on sludge used as manure.

2 Sample of sludge as collected from farmers did not contain alarming levels of Cd though Pb levels are high in a few samples

3 There were no untoward affects seen on the health of the people in the affected area.

B. CANCER AND XENOBIOTICS

1. *IN VIVO* ANTIMUTAGENIC EFFECT OF GINGER IN CARCINOGEN EXPOSED RATS

Investigators : Kalpagam Polasa, K. Nirmala and T. Prasanna Krishna

Duration : 1 year

Date of completion : Dec. 2003

INTRODUCTION

Many natural agents including fruits and vegetables are known to provide protection from different degenerative diseases including cancer by preventing damage to the cellular components. A group of non-nutrient chemical moieties have been discovered in diet which show antioxidant activities and are potential preventive agents against chronic diseases. Some frequently consumed spices in India and other Asiatic countries have been to claimed to exhibit antioxidant potentials. Effective antioxidants have been found in a number of frequently used spices and herbs like rosemary, sesame seeds, oregano, pepper, chilli, ginger and turmeric. Spices such as turmeric, ginger and onion which form an integral part of Indian and continental cuisines are known to contain certain bioactive phytochemicals in them.

Ginger rhizome (*zingiber officinale roscoe*) contains a rich source of biologically active constituents including the main pungent principles like gingerols, shogaols and paradols.

HYPOTHESIS

In the previous Annual report (2003), evaluation of antimutagenic potential of ginger was reported by carrying out an *in vivo* experiment in rats and quantifying urinary mutagens by Ames test with Salmonella typhimurium TA 98. The study demonstrated the antimutagenic potential of ginger. In order to support these observations, another tester strain namely Salmonella typhimurium TA 100 was used for evaluation.

STUDY DESIGN

Inbred male NIN/wistar rats aged 8-10wks were taken and divided into four groups containing six rats per group. The first group received standard laboratory diet.Group2,3,and 4 were fed with diet incorporated with ginger powder at 0.5%, 1% and 5% levels for a period of one month. The weekly food intake of animals were recorded and the body weights of all the groups were recorded at the beginning and end of the experiment. After one month of ginger feeding the animals were given injection of 5mg of B(a)P/rat in a single dose and 24hr urine was collected. Mutagensis were recovered by passing the urine through XAD-2 column. The urine extracts were used for carrying out the Ames test with S.typhimurium TA 100 and the assay was carried out both in the presence and absence of S_9 fraction.

RESULTS

a. There were no significant differences in weight gain between control and experimental animals at the end of the feeding period indicating that ginger containing diet did not alter the intake of diet.

b. Elevated no. of revertants (p< 0.001) were observed in urine extracts of carcinogen exposed rats (Table23). Similar observations were made in the presence of metabolic activation system (S_9)

Table 23. Inhibition of urinary mutagen levels in ginger fed rats

Presence/ absence of 9000 g. rat liver supernatant	No. of revertants (Salmonella typhimuruim TA 100)							
	Control		0.5% Ginger		1% Ginger		5% Ginger	
- S_9	- BP	+ BP	- BP	+ BP	- BP	+ BP	- BP	+ BP
	168 ± 7.7	352[a] ± 13.3	166 ± 13.8	291[b] ± 7.2	157 ± 11.3	226[c] ± 14.6	15.5 ± 11.8	189[d] ± 17.9
+S_9	239 ± 10.9	551[a] ± 14.8	233 ± 9.4	399[b] ± 14.5	222 ± 7.9	328[c] ± 12.8	223 ± 11.3	294[d] ± 13.6

Values are mean ± SD of 6 animals/group
Parallel comparisons : Values with different superscripts are significant at P < 0.001 by ANOVA

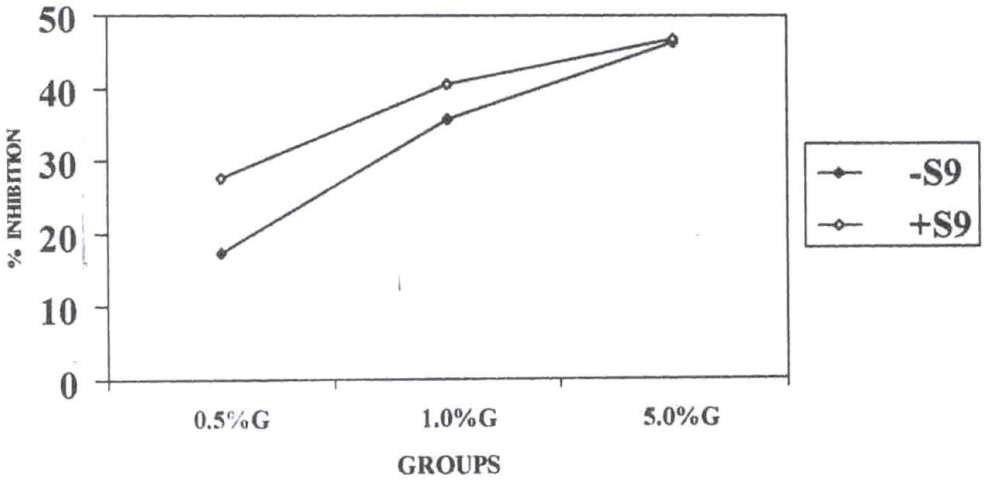
c. In the urine of rats prior fed with ginger for a month and treated with B(a)P a significant reduction (p<0.001) in the mutagenicity was observed as indicated by decreased no. of revertants. This observation was consistent at all the levels of ginger feeding as well as in the presence and absence of S9 mixture (Table 23).

d. In the absence of S9 the percentage inhibition with tester strain TA 100 was found to be 17.3,35.8 and 46.3 and in the presence of S9 it was 27.6,40.5 and 46.7 in the rats fed with ginger through diet at the levels of 0.5%, 1% and 5% respectively (Fig 20).

CONCLUSION

Feeding ginger to rats showed antimutagenic effect under in vivo as observed in Ames test using tester strain of Salmonella typhimurium TA 100.

Fig 20. Percent inhibition of urinary mutagens in ginger rats (TA 100)



2. ETHNOPHARMACOLOGICAL VALIDATION OF BIODYNAMIC COMPOUNDS IN TRADITIONAL MEDICINE

Investigators : Manjula, B. Dinesh Kumar and T. Prasanna Krishna

Duration : 1 Year

Date of completion : Dec. 2003

INTRODUCTION

Natural and synthetic antioxidants play a vital role in protecting cells and tissues against oxidative damage caused by free radicals. The natural anti-oxidants are receiving greater attention in view of their low toxicity and wider therapeutic potentials. Our earlier results showed that the water and water+methanol extracts of plants coded 2, 3, 4 and 5 were found to have good antioxidant activity as evaluated by battery of in vitro tests (Annual Report- 2002). It is known that oxidative stress plays a major role in chronic inflammatory disease like arthritis and plant extracts (4212, 3223) are conventionally used / reported for the treatment of arthritis. The findings of the in vitro results have also confirmed the antioxidant potentials of the preparations prescribed/reported traditionally.

HYPOTHESIS

To investigate the therapeutic potential of plant extracts 4212, 3223 in patients suffering from rheumatoid arthritis and correlate the efficacy with the antioxidant potential.

METHODOLOGY

A randomized double blind clinical trial was done in collaboration with Govt Ayurvedic College in 90 subjects suffering from Rheumatoid arthritis classified based on ARA (American Rheumatoid Association) criteria. All the formulations I, II and III (Test compounds 4212, 3223 and placebo) were packed in aluminum foil and the sachets were coded. Patients were recommended to take the drug for 40 days (1 sachet/day). The subjects were examined clinically and monitored for their biochemical, serological and hematological parameters before and after the treatment. The inflammatory conditions associated in rheumatoid arthritis was also measured by Algo functional sequence tests, ARA scoring and ESR before and after the treatment. The antioxidant activities (TBARS, Total Glutathione and Protein Carbonyls) were also evaluated at three time points i.e. before, mid and after the treatment.

RESULTS

a) There were no abnormalities in the routine haematological, biochemical and serological parameters before and after the treatment

b) However there was significant decrease (40-50%) of ESR levels in the treatment groups without much change in placebo treated group.

c) There was significant improvement in the clinical condition by 60-65% as measured by Algo functional sequence tests and scoring by ARA criteria in the treatment groups.

d) The antioxidant profile showed an increase in the total Glutathione levels (71.1-180 µg/ml plasma i.e. 120-150%) by 2 - 2.5 fold, decrease in protein carbonyl levels (3.94-2.4 nm/g protein i.e. 36-37%) by 0.6 fold in both the treatment groups. However group treated with 4212 alone showed decrease in TBARS (2.81-1.5 nm/g protein i.e. 46%) by 0.5 fold.

e) The results showed a possible correlation between the antioxidant potential and clinical outcome.

CONCLUSIONS

1) The antioxidant property as measured in vivo by oxidative markers viz. TBARS total Glutathione and Protein Carbonyls indicated potential antioxidant property of 4212 and 3223.
2) Conventionally prepared plant product formulations 4212 and 3223 posses (in vivo) antioxidant activity with leading to positive clinical outcome.
3) The observations indicate over all improvement in symptoms may be due to reduced oxidative stress and provides evidence for their use in traditional medical practice.
4) Further studies are planned in animals to demonstrate the mechanism of action.

3. DRUG CONSUMPTION PROFILE IN RURAL/URBAN ANDHRA PRADESH – A PILOT STUDY (PHASE II INTERVENTION)

Investigators : B. Dinesh Kumar, T. Prasanna Krishna, Anil Kumar Dube and B. Sesikeran

Duration : 1 Year

Date of completion : December 2003

Extension : 1 year

SPECIFIC REASONS FOR EXTENSION

To study the impact of intervention programme developed consequent to completing the pilot study in one region (Telangana) of Andhra Pradesh.

INTRODUCTION

In recent years, the use of drugs has considerably increased in almost all countries. Such a use, especially of irrelevant and unnecessary drugs not only escalates the cost on health but also can be harmful to the consumer under certain circumstances. The results of our study (Annual report 2001) have indicated existence of high injection rate (70-80%) with unnecessary use of drugs viz. antibiotics (27%), nutritional products (30%) etc, in addition with low rate prescription and dispensing time. Since past few decades, various agencies both at national and international level are making concerted efforts to develop an intervention programme to promote rational use of drugs and create an awareness on harmful effects of irrational use of drugs. (The study is being conducted as a part of India-WHO programme on essential medicines and sponsored by Delhi Society for Promotion of Rational Use of Drugs (DSPRUD)).

HYPOTHESIS

Illiteracy, lack of knowledge and other local considerations promote irrational use of drugs among majority of health seekers. Hence there exits a need to develop intervention programme. To create awareness on the concept of rational drug use with special emphasis on harmful effects of unnecessary use of injections, antibiotics, nutritional products. To pre-test and assess the impact of the intervention progamme on drug use profile specially at consumer level.

METHODOLOGY

A pilot study has been undertaken in a selected area of Andhra Pradesh in Nizamabad district , where the basic information on drug consumption profile has been collected earlier (AR 2001) from (10PHC, 4CHC & DH) in pre-tested schedules. This is followed by the display of intervention programme in PHC (7)/CHC (3)/DH(1) & schools from the respective villages etc. The data on drug consumption profile has been collected, compiled and analyzed for the following indicators before and after intervention programme using WHO guidelines.

1. Prescribing Indicators
2. Patients care indicators
3. Facility Indicators
4. Communication indicators

Intervention programme

The intervention programme consisted of (a) display of film titled *"Haridas-Tale of Medicines"* developed in a local folk form with catchy telugu songs for 36 minutes and (b) distribution of brochure concentrating the following messages:

1. Rationalizing the use of injections with specific reference to unwanted preparations and promotion of disposable syringes.
2. Rational use of antibiotics.
3. Quacks and their hazardous practices.
4. Side effects on use of wrong medication.

RESULTS

1. The results of the pre-intervention study was found similar to our earlier observations (annual report 2001) with high injection rate (60-70%), unnecessary use of antibiotics (30%), nutritional products(50%) etc. The literacy status was 20-30% in patients attending primary health centres.
2. In majority of the patients (60-70%) , there was a misconception on therapeutic benefits of injectables that it relieves the disease symptoms quickly.
3. The impact on the messages through film & circulation of brochures have been well received by school children (80%), patients (40-60%). Majority of the patients didn't understand the message on use of antibiotics resulting in a trend towards the quack practices and unnecessary use of injections causing side-effects.
4. The post intervention data has indicated the reduction in use of injectables(30%) nutritional products (20%), antibiotics (10%)etc. The impact could not be observed for antibiotics use suggesting a need for repeated intervention programme.

CONCLUSIONS

1. The intervention programme specially video display on the importance of rational use of drugs with special emphasis on reduction in Injection rates has yielded a very good response among children as compared to adult patients.
2. The study suggests that the repeated intervention programme can certainly promote Rational drug use practices. A study has been undertaken to study the effect of impact of repeated intervention programme on promoting rational drug use at one of the centres in Telangana.

VII. PRE-CLINICAL TOXICOLOGY

A. SERVICES ACTIVITIES

The Pre-clinical Toxicology Unit provides the following services:

1. Develop protocols for Pre-clinical studies as per DCGI/DBT/ICH guidelines for products like conventional molecules; recombinant DNA products, vaccines and herbal or drugs from indigenous systems of medicine.
2. Conducting pre clinical toxicology/ safety studies as per GLP principles (GLP accreditation awaited).
3. Histopathology services for tissues submitted by drug development/ safety testing organizations
4. Provides raining on an ad hoc basis for technicians and scientists working in this area.

B. RESEARCH ACTIVITIES

TOXICITY AND ALLERGENICITY EVALUATION OF RECOMBINANT HEPATITIS B VACCINE IN MICE AND GUINEA PIGS

Investigators : B. Sesikeran, B. Dinesh Kumar, A. Nadamuni Naidu, S. Hariharan, S. Kalyana Sundaram, T. Prasanna Krishna, P. Uday Kumar, SSYH Quadri and V. V. Annapurna, V.A Srinivasan, G. S. Reddy, D. Thiagarajan and L. Rajendra (Indian Immunological Limited)

Duration : 2 years

Date of completion : Nov. 2002

INTRODUCTION

Hepatitis B virus infection is a major health problem worldwide. Treatment options are limited and expensive. About 45 million people in India are infected with Hepatitis B and most are asymptomatic carriers. The disease may ultimately lead to cirrhosis, hepatic failure or hepatocellular carcinoma. Prevention by vaccination is the best option available.

Vaccine developed with recombinant DNA technology has high immunogenicity and provides good seroprotection.

The present study was undertaken to evaluate Toxicity & Allergenicity of recombinant Hepatitis B Vaccine, manufactured by M/s Indian Immunologicals Limited, Hyderabad, India, as per the schedule 'Y' of DCGI.

METHODOLOGY

Balb/C Mice and Guinea Pigs were selected, conditioned, and exposed to the test compound (with Recombinant Hepatitis B Vaccine) through intramuscular route at various concentrations (VC, 1X, 5X and 10X). The animals were observed for 40 days after the last exposure to record the following observations:

Routine Physical, Physiological examinations, Hematology, Clinical Chemistry, parameters, Gross necropsy, Histopathology and Immunopathology of major organs.

Statistical analysis

Data compiled and analysed for significance of difference between treatment groups and vehicle control.

RESULTS

The results of the study were as follows:

- No pre-terminal deaths,
- No significant treatment related effects on food intake, body weight, clinical signs, behavioural activity etc.
- No significant changes in leucocyte, erythrocyte, Differential leucocyte, Platelet counts and Hemoglobin in animals exposed to test compound at various concentrations of hematological parameters.
- There were no significant changes in blood glucose and protein levels as well as liver and kidney function tests.
- No specific test compound-induced pathological changes in the various organs were observed.
- No allergenicity reactions reported.
- No evidence of any Immunotoxicological effects

CONCLUSION

No specific abnormalities in physical, physiological, clinical chemistry, hemato-logical, pathological, immuno-toxicological, allergenicity profiles were recorded in mice and guinea pigs exposed to the test compound at various dose levels under the experimental conditions.

VIII. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

A. SERVICE ACTIVITIES

1. SUPPLY OF ANIMALS

When compared to the previous year, there has been a 16% increase in the breeding and 4% increase in the supply of animals during the current year. Animals of various species and strains amounting to 30471 were bred and 26157 animals supplied during the period. The income generated also increased by 24.2% generating Rs.24.1 lakh when compared to 19.5 lakhs in the previous year (Tables 24 & 25).

Like last year, the BALB/c mice colony in the barrier maintained facility showed minimum mortality of 0.14%. The maximum mortality was observed in NIH Hartley White guinea pigs, most of the mortality being during pre- weaning period. This could probably be due to stampede by the older animals. To overcome this problem the number of animals/ per trough is being by reduced.

The nude mice [NIH(s)] continued to show high mortality in the barrier maintained colony, which can be solved only by transferring all the animals to isolators. The paucity of sufficient number of isolators will be attended to very soon. In the conventional colony, WNIN rats showed a minimum mortality of 3.4%. Mutant rat strains like WNIN/Ob, GR-Ob and wild white rats continued to show high mortality rates of 19.1, 18.9 and 37.1% respectively, probably due to their increased susceptibility to infection, compared to other strains.

2. SUPPLY OF ANIMAL FEEDS

During the year under report, 22,201 kg of rat/mice feed and 2163 kg of guinea pig/ rabbit feed were supplied generating an income of Rs.15.97 lakhs. There has been an increase of 9% in the supply of feed and 27.8% increase in the income generated from this activity.

3. SUPPLY OF BLOOD AND BLOOD PRODUCTS

During the year, 425 ml of blood/sera from various species of animals were supplied to 5 different institutions on 18 occasions generating an income of Rs.25, 370/-. The Centre and the parent institution utilized 150ml of blood.

4. HEALTH MONITORING OF LABORATORY ANIMALS

As suggested during the Scientific Advisory Committee meeting of the Centre, attempts have been made to increase the representative number of samples of various species for health monitoring.

A total number of 644 samples from Conventional, Barrier maintained and Isolator bred colonies were screened during the year. The breakup is as follows:

Animals: - Mice - 209, rats - 122, g.pigs - 95, rabbits - 95, hamsters - 10,

Others: - Water -13, diet - 13, bedding - 13 and equipment - 71 (cages, laminar hood etc).

Additionally, lung swabs from personnel (3) working in the colony were also screened.

E.coli, Kl. pneumoniae, Listeria monocytogenes, Streptococcus spp., Staphylococcus spp. Bacillus spp. Corynebacterium spp. Sarratia liquifasciens, Pseudomonas spp. Proteus mirabilis, A calco var anitrat and Micrococcus spp, were the common bacteria encountered during the screening.

Additionally, Ectoparasites like mites were seen in the conventional and barrier maintained mice strains. Endoparasites like Syphasia obvelata was seen in almost all rats and hamsters had Giardia as well. Mostly these were seen in older animals. Viral screening is in progress and samples were collected for this purpose.

SICK ANIMALS FROM DIFFERENT COLONIES

As many as 95 clinically sick animals were reported from barrier maintained colony. Among the rats 16 belonged to WNIN, 32 to SD and 2 were Fischer. From mice, 23 were reported from BL57/6J colony, 10 from nude and 4 from rabbits, and 8 from guinea pigs. Many of these animals were having tumors, hair loss and some of them were suffering from middle ear infection and dermatitis. Appropriate samples were collected from tissues of these animals and microbiological and pathological examinations were conducted. Pathological lesions indicative of parasitical, viral and bacterial infections were observed in the tissues of these animals. However many of them were old animals.

5. HUMAN RESOURCE DEVELOPMENT

The Centre organized the following training courses:

i) 36th Annual Laboratory Animal Technicians' Course - Eleven candidates from various regions of the country successfully completed the course.

ii) Ad hoc Training on microbiological health monitoring was provided to two candidates from a pharmaceutical concern.

6. PRE-CLINICAL TOXICOLOGICAL WORK

Studies on PCT of Hepatitis B Vaccine were completed during the year. The centre provided BALB/c mice and NIH Hartley guinea pigs for this study. Indian Immunologiclals Ltd., Hyderabad, sponsored this study.

7. NEW INITIATIVE

National Animal Resource Facility at Genome valley, Turkapally.

The centre prepared a concept paper on the development of the above facility along with a brochure. The details were discussed in a meeting held at NIN on 17th March, 2004. The meeting was attended by the Secretary, Dept. of Health, Ministry of Health & Family Welfare (Centre), Director General, ICMR , Secretary, Dept. of Industry & Commerce, Govt. of A.P. and representatives from some of the pharmaceutical companies. The DG, ICMR presented the salient aspects of the proposed facility. It was concluded that Secretary, Dept. of Industry & Commerce, AP will assist in projecting this venture as a business model. Once this is ready, this will be further discussed with the concerned ministries and the industry.

Table 24. DETAILS OF BREEDING AND SUPPLY OF DIFFERENT SPECIES AND STRAINS OF LABORATORY ANIMALS (BARRIER MAINTAINED COLONY) DURING THE PERIOD FROM 01.4.2003 TO 31.03.2004

Sl. No	Species	Strain or Breed	Stock As on 1.4.2003	Total Number of animals							Balance as on 31.3.04
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied	Died	Disp	
1	Mouse	BALB/c An. N (inbred)	890	7788	8678	280	7212	7492	13 (0.14)	-	1173
		C57BL/6J (inbred)	261	2823	3084	21	2322	2343	105 (3.4)	-	636
		N:NIH(S) Nude (athymic) (inbred)	170	1037	1207	-	540	540	354 (29.3)	95 (7.9)	218
		C57 BL/6 nude (athymic)	3	-	3	-	-	-	3 (.0)	-	-
2	Rat	Wistar/NIN (inbred)	355	3721	4076	16	3356	3372	42 (10.9)	103 (2.5)	559
		SD (Sprague Dawley) (Outbred)	325	1246	1571	72	865	937	53 (3.4)	146 (9.3)	435
		Fischer 344 N (inbred)	161	88	249	12	120	132	24 (9.6)	44 (17.7)	49
3	G. Pig	N:HART (Hartley)	110	256	366	30	213	243	50 (13.7)	-	73
		Dunkin (Hartley)	130	302	432	15	230	245	47 (10.9)	-	140
		N:NIH (Coloured)	116	220	336	-	166	166	28 (8.3)	-	142
4	Rabbit	New zealand white	57	92	149	32	60	92	10 (6.7)	-	47
	TOTAL		2578	17573	20151	478	15084	15562	729 (3.6)	388 (1.9)	3472

Percentage of animals supplied to other Institutions: 85.8 %

NIN : 2.7 %

( ) Values are percentage of number of animals available in each species.

National Institute of Nutrition (ICMR)
Hyderabad 500 007
Website: http:/www.ninindia.org

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Sl. No	Species	Strain or Breed	Stock as on 01.04.03	Total Number of animals							Balance as on 31.3.04
				Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied	Died	Disp.	
1	Mouse	Swiss (inbred)	886	4910	5796	163	4593	4756	271 (4.7)	110 (1.9)	659
2	Rat	WNIN (inbred)	722	5782	6504	300	4641	4941	218 (3.4)	413 (6.3)	932
		WNIN/Ob-Ob (inbred)	497	247	744	85	-	85	155 (19.1)	123 (16.5)	381
		WNIN/GR-Ob	583	458	1041	69	-	69	194 (18.9)	292 (28.0)	486
		Wkyoto (inbred)	183	299	482	54	-	54	66 (13.7)	229 (47.5)	133
		CFY/NIN (inbred)	99	75	174	-	100	-	21 (12.0)	81 (46.6)	72
		Holtzman (inbred)	201	345	546	-	-	100	37 (6.8)	187 (34.3)	222
		Wild White	45	25	70	-	555	-	26 (40.0)	-	44
3	Hamster	Golden (inbred)	124	746	870	35	-	590	64 (6.2)	68 (7.8)	148
4	Monkey	Macaca mulatta (Rhesus)	24	-	24	-	-	-	-	-	24
5	Sheep		1	-	1	-	-	-	-	-	1
6	G.pig		1	8	9	-	-	-	5	-	4
7	Rabbit		12	3	15	-	-	-	9	-	6
TOTAL			3378	12898	16276	706	9889	10595	1066 (6.5)	1503 (9.2)	3112

Percentage of animals supplied to other Institutions: 76.7 %

NIN: 5.5 %

( ) Values are percentage of number of animals available in each species.

B. RESEARCH ACTIVITIES

1. MOLECULAR ANALYSIS OF WNIN/Ob - THE OBESE MUTANT STRAIN DEVELOPED FROM WNIN RAT COLONY MAINTAINED AT NCLAS

Investigators : M.Ananthaswamwarna Rao, N.V.Giridharan, P.Kondaiah (Indian Institute of Science, B'lr.)

Duration : 5 years

Date of completion : 2004

BRIEF BACKGROUND

WNIN/Ob is the mutant obese rat strain developed at NCLAS from the parental inbred Wistar stock (WNIN). The mutant strain has three distinct phenotypes i.e. lean (+/+), carrier (+/-) and the obese (-/-). The carriers and the obese phenotypes are characterized by the kinky tail.

The present project was undertaken to find out whether obesity in these rats is due to some of the well known gene mutation(s) which are shown in other rodent obese models especially leptin, its receptor, melanocortin, neuropeptide, Y (NPY) etc.

METHODOLOGY

1. CDNA from coding regions of leptin, leptin receptor, melanocortin receptor and neuropeptide Y were amplified by RT-PCR from RNAs isolated from the tissues of three phenotypes (+/+, +/- and -/-) of WNIN/Ob rat strain.
2. DNA sequences of amplified products of leptin and leptin receptor genes were done using Sanger's chain termination method and compared by Blast search.
3. Northern blot analysis was done using RNA's isolated from the brain of the three phenotypes to study the expression of leptin using ^{32}P labelled leptin probe.
4. Semi quantitative RT, PCR analysis was done to establish the differences in the steady state levels of mRNAs for leptin receptor, melanocortin receptors, and NPY.

RESULTS

1. With regards to leptin gene the PCR amplified products of the three phenotype, did not show any difference from the known leptin sequence in the genebank by BLAST search. It can be safely concluded that there is no defect in leptin gene in these mutants.
2. Northern blot analysis of RNAs from the brains of the three phenotypes showed increased expression of leptin gene in (-/-) phenotype.
3. Though several primers (coding the entire region of the leptin receptor gene (Ob-Rb) to generate PCR products of 500 bp), spanning the entire leptin receptor gene were employed, the DNA sequences of each of the PCR product did not reveal any mutation compared to the leptin receptor sequence reported in the gene bank.
4. Further semi-quantitative RT-PCR analysis of isoforms of leptin receptor forms, also did not reveal any difference in the steady state levels of m-RNA;s, showing no change in the promoter region of the gene. These data thus clearly rules out a molecular defect in the leptin receptor gene of the WNIN/Ob rat.

5. The amplified product of MCR-4 gene was of expected size in all the phenotypes and the MCR-4 expression was found to be high in lean in comparison with the obese phenotype.
6. Sequence analysis of amplified product of NPY gene from lean and obese phenotypes did not show any difference.

CONCLUSIONS

It is evident from the above results that in contrast to the established rat models, obesity in the WNIN/Ob rats is not due to changes in some of the known gene(s) of obesity i.e. leptin and leptin receptor. The normal DNA sequence of leptin receptor gene does not explain our earlier observations of low receptor binding. Therefore, detailed analysis of leptin receptor gene expression and leptin receptor binding studies need to be carried out to explain this discrepancy. MCR-4 receptors also need to be studied in depth to understand the nature of obesity in these mutants.

2. ESTABLISHMENT OF BASE LINE VALUES OF BODY AND BLOOD PRESSURE IN DIFFERENT SPECIES OF LABORATORY ANIMALS MAINTAINED AT NCLAS, NIN

Investigators : N.Harishankar, R.Subramanian, S.Kalyanasundaram and N.V.Giridharan

Duration : 5 years

Date of completion :

BRIEF OUTLINE

NCLAS houses different species and strains of laboratory animals for biomedical research both for in-house use as well as outside supply. It was decided to generate base line data on some important physiological and biochemical parameters of these laboratory animals. We recently acquired a biomedical instrument to measure Total Body Electrical Conductivity (TOBEC), which is useful for analyzing body composition of laboratory animals, non-invasively. The center has also an equipment to measure blood pr essure, an auto track system to measure activity and oxymax respirometer for basal metabolic rate etc., During the year body composition and activity patterns of the three rat strains maintained in the facility viz., WNIN, Sprague Dawely (SD) and F-344N were measured along with estimation of some serum/plasma clinical chemistry parameters.

METHODOLOGY

Animals

Weanling rats of WNIN, SD and F-344N strains (12 ?+12 ?) housed under standard experimental conditions were used for the studies.

Body composition

Body composition of the animals was determined using *TOBEC* small animal body composition analysis system (EM - SCAN/TOBEC Model SA – 3000 Multi detector), which measures total body electrical conductivity (E) to arrive at the composition.

The difference between the impedance measured when the animal is inside the electromagnetic field and when the chamber is empty is an index of the total electrical

conductivity (E) of the body, which in turn is proportional to the animal's lean body mass. WNIN, SD and F-344N rats at different ages (50,100,150,200 and 250 days) were analyzed for progressive changes in body weight and fat content of the body. Lean body mass, total body fat were estimated and parameters like total body water, total body sodium and total body potassium were derived mathematically.

Spontaneous Loco motor Activity

The detailed activity patterns, which include distance traveled (DT), resting time (RT), stereotypic time (ST), ambulatory time (AT) were quantified using a Columbus Activity cage. The activity measurement was made for a period of 4 hours each during "Light" and "Dark" hours of the day (09.00 -13.00 and 18.00-22.00 hours respectively). The activity was recorded in adult male and female rats (200 days).

Clinical chemistry

Blood was collected from males and females of three strains at 200 days of age following 17 hours fast; plasma samples were separated and stored at - 20 °C until analyzed. Glucose, total protein, albumin, urea, creatinine, calcium, phosphorus, total bilurubin, SGOT, SGPT, gama glutamyl transpeptidase, alkaline phosphatase, choloesterol, HDL cholesterol and triglycerides were measured in plasma using Schiapparelli biosystems inc. autoanalyzer.

RESULTS

Body composition

All the three strains, i.e., WNIN, SD and F-344N rats showed progressively higher body weights and lean body mass (LBM) with increase in age. Derived parameters like fat, fat%, FFM, TBW, TBK and TBNa were significantly different among the three strains and also between sexes ($P<0.001$). SD rats (both male and females) had higher weights and LBM followed by WNIN and F-344N at all the ages studied.

S.D females showed higher body weights than males at 50 days of age, unlike the other two strains (WNIN, F-344) where in the males showed higher weight than females at that age. However, at 100 days onwards the males weighed more than the females in all the three rat strains. Though the total body fat was more in SD rats, when expressed as percentage fat, WNIN male rats showed significantly higher values.

Activity

All strain of rats irrespective of sex showed significantly higher distance traveled (DT) during night time when compared to daytime ($P < 0.001$). Among the strains, total DT is high in F-344N followed by SD rats and WNIN rats.

In contrast resting time (RT) in all the three strains was significantly higher during day compared to nighttime and there were significant differences between the sexes. Male rats showed maximum RT and this was highest for WNIN followed by SD and F-344N rats in that order. Mean ambulatory time (AT) was high in F-344 rats compared SD and WNIN rats. ($P<0.001$). This was high during nighttimes compared to daytime in all the three strains and higher in females compared to males in WNIN and F-344 rats. However no significant difference was seen between male and female SD rats. Mean stereotypic (ST) was highest in SD rats followed by WNIN and F-344 rats and between the sexes, females had higher activity than males.

Clinical chemistry

Glucose levels were significantly high in SD rats, followed by F-344 and WNIN rats. No significant differences were seen between sexes. Total protein, albumin and urea levels were higher in F-344N males than WNIN and SD rats, while WNIN female rats showed higher values than F-344N and SD female rats ($P<0.001$). F-344N rats had the highest triglyceride values followed by WNIN and SD rats. Between sexes males had higher values when compared to females ($P<0.001$). Cholesterol and HDL cholesterol levels were high in males compared to females ($P<0.001$). Among the strains, SD rats had higher cholesterol values than WNIN and F-344N rats ($P<0.05$). Alkaline phophatase and total bilurubin levels were significantly high in males compared to females in all the three strains ($P<0.001$). The strains, WNIN and F-344N rats had higher values compared to SD rats ($P<0.001$). No significant differences in creatinine, GGT, calcium, phosphorus, SGOT and SGPT were seen between three strains and sexes.

CONCLUSIONS

The study showed significant differences between the three strains of rats, in terms of body composition, physical activity and serum clinical chemistry. Though body weight as well as total body fat was more for SD rats, the amount of fat for the given body weight was higher in WNIN. In terms of activity, WNIN rats showed least nighttime activity and its resting time in daytime was also higher. F-344 had lesser body weight, lesser fat and it reflected on its higher nighttime activity and also less resting activity during the day. Among the strains, SD rats showed higher glucose and cholesterol values.

INSTRUMENTATION SERVICES

The department has participated and contributed to the Institution and Manpower building activities in various ways. The department activities include maintenance and repair of Electrical, Electronics, Electromechanical, Ref & A/C equipment. While equipment maintenance remains the priority of the department, training of staff is also considered important to prevent equipment failure and optimum utilization.

The first and foremost activity being the development of suitable work force to deliver the expected output. The staff of the department have themselves offered training to the various staff of the institute on the operation of various equipment already in use and also those which were installed during the period, besides getting themselves trained. Preference has been given to quality control in Instrumentation and a lecture was delivered by the Head of the Department. Up gradation of existing knowledge and elevation of expertise is another area wherein staff were deputed to seminars and workshops organized by various organizations, national and international.

In order to provide a conducive work atmosphere, instruments rooms were given a face lift by providing false – roofing, flooring etc. Proposals to provide modular furniture are in progress.

Documents were prepared for equipment requirement with justifications to be placed before the SAC. Technical specifications were prepared and placed before the Technical Experts Committee of the Institute.

The Technical Bids of various categories of equipment were evaluated and reported to the Technical Expert Committee for its evaluation. Steps were taken to regulate usage of equipment in various rooms, with equipment details listed therein, for the convenience of staff.

The following facilities were modernized/ established during the period:

1. Establishment of Modern Biology Laboratories.
2. Establishment of International Centre for estimation of Vit.A for Dry Blood Spot with HPLCs, Spectrophotometers, Sonicators etc.
3. Modification and modernization of Director's secretariat.
4. The Committee Hall
5. Class Room II in Gopalan Block.
6. Establishment of a HPLC analytical facility and enhancing the work out put by installing Auto Sampler.
7. Establishment of GM Food Analytical facility.
8. Establishment of equipment facilities at Primate Centre.

Participation in Training Programmes of the Institute:

1. Master of Science Applied Nutrition.
2. Endocrinological Techniques.
3. DXA – Muliticentric –Training Programme.
4. Inplant training for participants from Advanced Training Institute, Ramanthapur, Hyderabad.

SCIENTIFIC ACTIVITIES

- Symposium on Body composition studies organized by NSI on 12th and 13th December 2003.
- Delivered a lecture on "Comparative evaluation of Body composition methods".
- Organization of Audio –Visual facilities for NSI at the Open Air Theatre.
- SAC meetings.

Seminar Club, Journal Club activities, Seminars were organized on the following topics for the information and benefit of staff by calling experts in the following techniques:

ICPMS, LC-MS, Micro Array Detection, Laser desorption techniques etc.

Staff deputed for conferences and workshops

Dr.R.Subramanian, Dy.Director:

Seminar on Drinking water solutions using ICPMS, LC-MS technologies at Mumbai on 16th December 2003 conducted by Agilent Technologies, Mumbai.

Mr.R.Chaugule, T.O.

Attended seminar on "Speed and Sensitivity new technologies on GC & GC-MS", conducted by M/s Niulab and Thermo Finnigan on 2nd December 2003 at Hyderabad.

Mr.Rajamouli, T.O

Seminar attended on "Speed and Sensitivity new technologies on GC & GC-MS", conducted by M/s Niulab and Thermo Finnigan on 2nd December 2003 at Hyderabad.

Seminar attended on "HPLC troubleshooting and validation" conducted by M/s Chromline Equipment(I) P.Ltd on 30th September 2003.

Mr.V.Satish Babu, T.O

Attended Workshop on Agilent 1100 series HPLC System on 1st September 2003 conducted by Agilent Technologies, Hyderabad.

Mr.B.Ramulu, T.O.

A two days programme on Energy Auditing and efficient operation and maintenance of Air-conditioning and Refrigeration Systems on 30th & 31st July 2003 at Delhi.

Mr.K.Sreenivasa Rao,T.A.

Attended seminar on "Speed and Sensitivity –New Technologies on GC & GC-MS, conducted by M/s Niulab and Thermo Finnegan on 2nd December 2003 at Hyderabad.

Mrs. L. Vijaya Durga, Technician

National Seminar on HPLC organized by Association of American Oil Chemists at IICT, Hyderabad on 28th & 29th April 2003.

Attended Workshop on Agilent 1100 series HPLC System on 1st September 2003 conducted by Agilent Technologies, Hyderabad.

Maintenance of Equipment

1. To regulate use of equipment, log books and reservation charts are maintained.
2. Service calls are regulated through entry of complaints in the complaints register.
3. Calls are also attended instantaneously as per needs.
4. Creation of uninterrupted power supply facilities are done to meet the demands.
5. Spare parts are procured as and when needed.
6. Maintenance of stock and consumables.
7. Preventive maintenance.
8. When servicing is found uneconomical, recommendations are made for condemnation.
9. Electrical-Transformer reconditioning and earth resistance maintenance are carried out every year in our Sub-Station.

No. of complaints received : 629
No. of complaints attended & completed : 611
No. of complaints pending :18

The following instruments were installed and validated during the period:

S.No	Name of the Equipment	Make	Model
1.	HPLC –2nos.	Thermofinnigan	Isocratic and Binary
2.	HPLC	Shimadzu	Quarternary gradient 10VP Series.
3.	4 capillary DNA Sequencing System	Applied Bio Systems	ABI Prisam 3100
4.	RT PCR	Bio-Rad	I Cycler
5.	FPLC	Amersham	Aktaprime
6.	Protein Purification System	Amersham	
7.	Spectrophotometer	Thermospectronic	330E
8.	Spectrophotometer	Perkin Elmer	EZ201
9.	Spectrophotometer	Shimadzu	SP-65
10.	Spectrophotometer	Hitachi	U2800
11.	Gel Doc System	Bio-Rad	Geldoc-2000
12.	Gas Chromatograph	Varian	3800
13.	Spectroflourimeter	Varian	Cary Eclipse
14.	Multimedia LCD projector	Panasonic	PTLC 25
15.	Capillary Electrophoresis System	Bio-Rad	Bio-focus 3000
16.	Spectrophotometer cum plate Reader	Molecular Dynamics	Spectramax Plus
17.	CO2 Incubator	Forma and Hereaus	Forma Stericycle 371 and Heracell 240
18.	Balances	Sartorius	BL3
19.	Microscopes	Nikon	TE2000U
20.	Lead Analyser	TEA	
21.	Water Purification System –3nos.	Labconco-2 Millipore	MilliQ ICPMS
23.	Barcode System	Bartronics India Ltd.,	M-90
24.	UPS 5 KVA –3nos.	Power one Electronics	
25.	Ultra Low Temp. Deep Freezer	Forma	991
26.	Table Top Ref.Centrifuge	Sigma	4k-15
27.	Polygraph –modules 1.Stimulator module 2. Electromyogram Amplifier	Biopac	STM100C EMG 100C

LIBRARY AND DOCUMENTATION SERVICES

Library continued to cater to the documentation and information needs of the Institute and other research organizations, home science and medical colleges. The Library has played a key role in reference activities by offering information services like MEDLINE Searches. ProQuest Searches, and other on-line retrieval activities using the LAN network. Library continued to participate in exchange of data and information using the URL < http://Groups.yahoo.com/Goup/ICMRLibrarians>.

Automation activity (Cataloguing) using the ISIS and LIBRIS softwares has been continued during the year, and a total of over 15000 records which have been retro-converted using the LIBRIS software.

The following Hardware is Procured for Library for Dessiminating the information expeditiously.

1) Two P4 (Compac) Computers.
2) i) Barcoding label Printer.
   ii) Barcode laser Scanner
   iii) Portable programmable Data Terminal.

The Library has continued to provide an excellent Photostat support to the scientists, technical as well as to the administrative staff. Resource Sharing and User-Education Programmes etc are continuously being undertaken by the Library. Institute's scientific papers going in for publication in Journals etc., are being routed through the Library and a data-base of the published papers is also made accessible through on-line services.

During the period a Total of 2209 ProQuest ML Full Text database Searches were made and there is overall 265% increase in its usage.

British library Institutional membership is renewed for 2003 and Corporate Membership for "Universities Federation for Animal Welfare " UK for the year 2003, has also been taken out during the year under report.

The following library services were expanded as detailed below.

1. NEW ADDITIONS

Books	. . . . .	86
Reports	. . . . .	354
Journals(New Subs.)	. . . . .	
Thesis / Dissertations	. . . . .	11
Microforms	. . . . .	55
CDROMS (MEDLINE)	. . . . .	40

2. OTHER ACTIVITIES

Journals Bound	. . . . .	610
Visitors using the Library	. . . . .	4622

Circulation of Books/Journals etc.	. . . . .	2388
MEDLINE Abstracts provided	. . . . .	4096
No. of E-mails sent outside	. . . . .	198
No. of E-mails received	. . . . .	1038
Photocopying (No. of pages)	. . . . .	5,91,545
Number of Annual Reports mailed	. . . . .	626
No. of Books/Journals received on	. . . . .	35
Inter Library Loan		
No. of Duplicate Journals sent out	. . . . .	200
No. of INTERNET Searches provided	. . . . .	252
No. of Reprints sent	. . . . .	180
ProQuest Full Text Database searches provided	. . . . .	33

3. TOTAL LIBRARY COLLECTIONS

Books	. . . . .	15,218
Journals (Bound Volumes)	. . . . .	27,118
Journals subscribed for 2002	. . . . .	247
Journals received (Gratis/Exchange)	. . . . .	285
Microforms (Microfiche)	. . . . .	1072
Slides	. . . . .	277
Reports	. . . . .	10,727
Reprints	. . . . .	3,07,576
Theses & Dissertations	. . . . .	345
MEDLINE CDROM Discs	. . . . .	168
Current Contents on Diskettes with Abstracts	. . . . .	664
ProQuest (Ful Text E-Journals) on CDROMS	. . . . .	410

Ph.D PROGRAMMES

Ph.D. Awardees

Research Scholar/ staff	University	Year	Title of thesis
1. Raghu P.	Osmania	2003	Characterization and significance of TTR_2
2. Radhika M. S.	Osmania	2003	Effect of food based vitamin A supplementation during pregnancy on maternal and child health

Research scholars registered for Ph.D.

Research Scholar/ Staff	Title of the project	Guide
1.Anil Kumar Dube (1993)	Nutritional education for urban adolescents: Use of social marketing principles in communication	Dr. Mohan Ram, M.
2. Rajendraprasad M.P. (1997)	Nitrosamines and its - relevance to cancer in India	Dr.Kamala Krishnaswamy
3. Nirmala K. (1999)	Antigenotoxic potential of ginger	Dr. Kalpagam Polasa
4. Pratima Rao (1999)	Multicentric study on intake of food colours	Dr.Ramesh V Bhat
5. Vijayalakshmi A. (2000)	Role of nutrition in modification of apoptosis	Dr.M.Raghunath/ Dr.B.Sesikeran
6. Saravanan N. (2000)	Effects of dietary alteration of n-6 and n-3 polyunsaturated fatty acids on insulin resistance, structure and function of adipocytes	Dr.Ghafoorunissa
7. Jeyakumar S.M. (2000)	Studies on food intake regulation and obesity in WNIN/Ob and WNIN/ GR-Ob rats	Dr. Vajreswari, A.
8. Rita Saxena (2000)	Role of food processing on antioxidant activity and development of recipes with high antioxidant activity	Dr. Kamala Krishnaswamy

9.Sreedhar B (2000)	Iron and Zinc interactions site of absorption	Dr.Madhavan Nair K
10. Venu L. (2001)	Foetal metabolic programming for insulin resistance: Role of maternal dietary micronutrients	Dr. Raghunath, M.
11. Satish Kumar M (2001)	Molecular chaperone function of α– crystallin	Dr. Bhanuprakash Reddy G.
12. Krishna Kumari Menon (2001)	Positive Deviance in child nutrition	Dr.Vijayaraghavan, K.
13. Manjula T. (2001)	Ethno-pharmacological validation of biodynamic compounds in traditional medicine	Dr. Dinesh Kumar, B.
14. Aruna B. (2002)	Biophysical characterization of resistin	Dr. Nasreen Z. Ehtesham
15. Haseeb A. (2002)	Understanding the mechanism of action of PPARg as a link molecule b between obesity, type 2 diabetes and CHDs	Dr. Nasreen Z. Ehtesham
16. Uma Devi A. (2002)	Study of energy metabolism in WNIN obese rat mutants	Dr.Giridharan N.V.
17. Kiran Kumar B. (2002)	Genetic typing of WNIN/Ob and WNIN/GR-Ob strains using microsatellite markers	Dr.Giridharan N.V.
18. Anil Kumar (2002)	Molecular chaperone function of α–crystalline under hyperglycemic conditions: Modulation by dietary factors	Dr.Bhanuprakash Reddy G.
19. Megha Saraswat (2003)	Screening of aldose reductase inhibitors and antiglycating agents from dietary sources and assessing their anticarcinogenic potential	Dr.Bhanuprakash Reddy G.
20. Mrudula T. (2003)	Charecterisation and significance of a novel fatty acid elongase of the eye lens	Dr.Bhanuprakash Reddy G.
21. Mr. Prasahanth B. (2003)	Role of scavenger receptors class B-1 (SR-B1) in reticulocyte differentiation, absorption of fat and fat soluble vitamins (vitamin A) and female infertility using WNIN/Ob rat model	Dr.Vajreshwari A.

Research Scholar/ Staff	Title of the project	Guide
22. Durga Kishore Y. (2004)	Role of maternal trace elements inthe development of insulin resistance in adult life	Dr.Raghunath M.
23. Vijay Kumar V. (2004)	The role of specific nutrients on the pancreatic progenitors/ stem cell specific to ductal epidelial cell	Dr.Vijayalakshmi V.
24. Subba Rao G.M (2004)	Approaches to Nutrition communication: A comparative study of effectiveness (Univ. of Hyderabad)	Prof. Vinod Pavarala

PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS

1. Dr. P. Suresh
Assistant Director

NIH Fellowship Programme on Management of primate colonies (SPF and Conventional) and health screening requirements for experimentation under ABSLS conditions, at National Primate Research Centre (NPRC), University of Washington, Washington, USA, for one year, under NCRR/NIH grant (15th April 2003 – 31st March, 2004).

Training on Laboratory Animal Sciences Individual Species conducted by University of Washington (1st May 2003 to 18th June 2003)

2. Dr. G. Bhanuprakash Reddy
Senior Research Officer

Post Doctoral Fellowship Programme, at University of Michigan, Kellog Eye Centre, U.S.A (April – July, 2003).

3. Dr. S. Chennaiah
Technical Officer

XII Workshop on Vitamin D at Maastrich, The Netherlands (July 6-10, 2003)

4. Dr. Ramesh V Bhat
Deputy Director (Sr. Grade)

Consultancy Assignment as TCDC Expert on Food Analysis (Mycotoxins) in Ulaan Baator, Mongolia (Aug.7-23, 2003).

3. Dr. N. V. Giridharan
Deputy Director

Discussion on Indo US Collaborative project on "Localisation and cloning of obesity gene in (WNIN/Ob) mutant rats, at Rockefeller University, Howard Hughes Medical Institute, New York, USA (Aug.14-Sept.14, 2003).

4. Dr. Ghafoorunissa
Officer-in-Charge

Palm Oil International Conference (PIPOC) 2003, held at Malaysia (Aug. 24-28, 2003)

5. Dr. B. Sivakumar
Officer-in-Charge NIN/FDTRC

Crop Meeting at International Rice Research Institute (IRRI), Manila Phillippines (Oct. 6-8, 2003).

6. Dr. P. Suresh
Assistant Director

International Council for Laboratory Animal Sciences Meeting organized in conjunction with American Association for Laboratory Animal Science Annual Conference, Seattle (Oct. 12-16, 2003).

National Conference on Specific Pathogen Free primate facilities, held in Seattle (Oct. 21, 2003).

Non-Human Primate models for AIDS research conference held in Seattle, USA (Oct. 22-25, 2003)

7. Dr. B. Sivakumar
Officer-in-Charge NIN/FDTRC

1st Asia Region Workshop on Functional Foods organized by the International Life Sciences Institute (ILSI) Southeast Asia Region, to be held at Kuala Lumpur, Malaysia (Oct. 22-23, 2003).

8. Dr. C. Vijayakumar Reddy
Technical Officer

First Technical International Conference on Polyphenols and Health held at Vichy, France (Nov. 18-21, 2003)

9. Dr. M. Raghunath
Assistant Director

First Annual World Congress on the Insulin Resistance Syndrome, at Hilton Universal City, Los Angeles, CA, USA (Nov. 21-22, 2003).

10. Dr. B. Sivakumar
Officer-in-Charge NIN/FDTRC

Pre-conference Symposium and Workshop on "Biotechnology-Derived Nutritious Foods – Challenges and Opportunities in Asia", at Bali, Indonesia (Feb. 29 - March 1, 2004).

Workshop on "Food Consumption and Dietary Exposure Assessment" organized by the ILSI East Asia Region, at Bali, Indonesia (March 4, 2004).

WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT THE INSTITUTE

1. The 36th MSc (Applied Nutrition) Course (16th June 2003 to 15th March 2004).

2. 36th Junior Laboratory Animal technicians Training Course (LATTC) (June 16 – 30th July, 2003).

3. Two day interactive programme on Research Methodology for prospective guides of research scholars, organized by NIN & NTR University of Health Sciences, Hyderabad (July 9-10), 2003.

4. One day training programme to orient on full text electronic databases Proquest and Ovid to the librarians of all ICMR institutions (July 26, 2003).

5. Adhoc training programme for two WHO fellows from Bhutan, in the field of Food Chemistry (July 7-Aug.1, 2003).

6. Training programme for the staff of NNMB Uttar Pradesh unit on the methodology of assessment micronutrient deficiencies and standardization in the rural population (July 21-Aug.1, 2003)

7. Meeting of the Pre-SAC and Scientific Advisory Committee of NIN/FDTRC/NCLAS (Aug. 4-6, 2003).

8. Training programme for the staff of NNMB unit of Karnataka, Gujarat on the methodology, assessment of micronutrient deficiencies and standardization using the procedures (Aug. 11-29, 2003).

9. Training for the officers of Food and Nutrition Board on "Training and capacity building in the field of Nutrition Monitoring and Surveillance" (Aug. 18-22, 2003).

10. XXXII Annual Training Course on Endocrinological Techniques and their Applications (Aug.18-Sept.30, 2003).

11. Ad-hoc training programme for Ms.Daphna Dror of USA at the institute for a period of nine months on the Fellowship support of Rotary International Organization, USA. Training in the field of Community studies for a period of four months (11th August – Nov. 2003).

12. National Nutrition Week celebrations (Sept. 1-7, 2003).

13. Training programme for the officials of DMRC, Jodhpur, on the methodology of diet and nutritional assessment (Sept. 15-26, 2003).

14. Training programme for the staff of South Central railway of Guntakal, on the methodology of assessment of diet and nutritional status and perception of diabetic diet (Oct. 13-17, 2003).

15. ICMR-WHO Workshop on "Intellectual Property Rights (IPR) and WTO Issues", held at the Institute (Oct. 16-17, 2003).

16. World Food Day Celebrations (Oct. 14-16, 2003).

17. Vigilance Awareness Week (Nov. 1-7, 2003).

18. ICMR Foundation Day Celebrations (Nov. 2003).

19. XXXV Annual Meeting of the Nutrition Society of India (Dec.12-13)..

20. One-Day Orientation Programme on Nutrition for High School Biology Science Teachers in association with Confederation of Voluntary Associations, Hyderabad. (Jan. 6, 2004).

21. Workshop on "Identification of outcome indicators of supplementary nutrition programmes and development of strategies for evaluation of programmes in Andhra Pradesh", in collaboration with Department of Women Development and Child Welfare, Government of Andhra Pradesh (Jan. 13, 2004).

22. XXXXI Post-Graduate Certificate Course in Nutrition (Jan.5 – March 12, 2004).

23. A two day interactive programme on Research Methodology for prospective guides of research scholars organized by NIN & NTR University of Health Sciences, Hyderabad (Feb. 24-25, 2004).

24. Training and Capacity Building for Food and Nutrition Board Officials in the field of Nutrition Monitoring and Surveillance System (March 8-12, 2004).

25. Training Course on "Techniques for Assessment of Nutritional Anaemias" (March 15-26, 2004).

26. Release of the "Final Report of IDD Survey by Sri J.V.R. Prasada Rao, IAS, Secretary, Ministry of Health and Family Welfare, Government of India. Prof. N.K. Ganguly, Director-General, ICMR, Dr.B.K.Tiwari, Adviser (Nutrition), DGHS participated (March 17, 2004).

27. Discussion on establishment of National Animal Resources Facility at Hyderabad – A Joint Venture of Government and Private Sector (March 17, 2004).

28. Inauguration of NIN Website by Sri J.V.R. Prasada Rao, Secretary, Ministry of Health and Family Welfare, Govt. of India (March 17, 2004).

29. Inauguration of National Facility for Dry Blood Spot (DBS) Technology for estimation of vitamin A by Prof. N.K. Ganguly, Director-General, ICMR (March 17, 2004).

SERVICES RENDRED TOWARDS INCOME GENERATION

1. PATHOLOGY SERVICES

During the year, a total income of Rs. 4,05,730/- was generated from various institute preclinical toxicology projects and surgical pathology and cytology samples.

2. FOOD ANALYSIS

A total amount of Rs. 1,07,500/- was generated from the analysis of foods for proximate composition, trace mineral and heavy metal content.

3. TRAINING PROGRAMMES

By admitting 6 unsponsored private candidates to the three regular training courses, a total of Rs. 55,000/- was generated

SCIENTIFIC PUBLICATIONS - 2003

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Aralappa N, Balakrishna N, Sharad Kumar, Brahmam GNV, Vijayaraghavan K : Diet and nutritional status of rural elderly in India. Journal of Nutrition for the Elderly. 22(4); 35-51, 2003.

2 Aruna B, Ghosh Sudip, Singh AK, Shekhar CM, Srinivas V, Radha Chauhan, Nasreen Z. Ehtesham : Human recombinant resistin protein disulfide linkages. Biochemistry. 42(36): 10554-10559, 2003.

3. Bamji MS, Bhat RV : Nutrition is the key to health and nation's development. Current Science. 84 (12): 1501-1503, 2003.

4. BhanuPrakash Reddy G, Khandare AL, Yadagiri Reddy P, Shankar Rao G, Balakrishna N, Srivalli I : Antioxidant defense system and lipid perioxidation in patients with skeletal fluorosis and in fluoride-intoxicated rabbits. Toxicological Sciences 72: 363-368, 2003.

5. Bhaskaram P, Balakrishna N, Radhakrishna KV, Kamala Krishnaswamy : Validation of hemoglobin estimation using hemocue. Indian Journal of Pediatrics. 70:25-28, Jan'2003.

6. Bhaskaram P, Hemalatha R, Narayana Goud B : Expression of messenger ribonucleic acid and production of cytokines in children with malnutrition. Nutrition Research. 23 : 367-376, 2003.

7. Bhaskaram P, Nair KM, Balakrishna N, Ravinder P, Sesikeran B : Serum transferrin receptor in children with respiratory infections. European Journal of Clinical Nutrition. 57 (1): 75-80, 2003.

8. Bhat RV : Nutrition goals for Asia-Vision 2020: Report of the IX Asian Congress. Asia Pacific J Clin. Nutr. 12(3):385-386, 2003

9. Binata Nayak K, Nair KM : In vitro bioavailability of iron from wheat flour fortified with ascorbic acid, Edta and sodium hexametaphosphate, with or without iron. Food Chemistry. 80:545-550, 2003.

10. Gorstein Jonathan, Bhaskaram P, khanum Sultana, Hossaini Reza, Balakrishna N, Goodman Tracey S, deBenoist Bruno, Kamala Krishnaswamy : Safety and impact of vitamin A supplementation delivered with oral polio vaccine as part of the immunization campaign in Orissa, India. Food and Nutrition Bulletin. 24(4): 319-331, 2003.

11. Krishnaiah YSR, Veer Raju P, Dinesh Kumar B, Satyanarayana V, Karthikeyan RS, Bhaskar P : Pharmacokinetic evaluation of guar gum-based colon-targetted drug delivery systems of mebendazole in healthy volunteers. J. Controlled Release. 88:95-103, 2003.

12. Mallikharjuna Rao K, Laxmaiah A, Ravindranath M, Venkaiah K, Hanumantha Rao D, Brahmam GNV, Vijayaraghavan K : Diet and nutrition during drought in Western Rajasthan, India. J. Human Ecology. 14(3): 153-158, 2003.

13. Pratima Rao, Bhat RV : A comparative study on the synthetic food colours usage in foods procured from urban and rural areas of Hyderabad. Nutrition and Food Science. 33(5):230-234, 2003.

14. Radhika MS, Bhaskaram P, Balakrishna N, Ramalakshmi BA : Red palmoil supplementation: A feasible diet-based approach to improve the vitamin a status of pregnant women and their infants. Food and Nutrition Bulletin. 24(2): 208-217, 2003.

15. Raghu P, Ravinder P : A new method for purification of human plasma. Biotech. and Appl. Biochem. 38(1): 19-24, Aug'2003.

16. Ramulu P, Udayasekhara Rao P : Total, insoluble and soluble dietary fiber contents of Indian fruits. J. of Food Composition and Analysis. 16(6): 677-685, 2003.

17. Roopa Ravikumar, Vijayapushpam T, Grace Maria Antony : Social factors responsible for fertility decline in Andhra Pradesh: A case study approach. Perspectives in Education. 19 (4): 213-220, 2003.

18. Sampathachary K : Design and development of a national information system in nutrition science for India (NISNUTS) : A study. Medical Library Association of India (MLAI) Bull. 60(1): 30-35, 2003.

19. Sitarama Raju KVV, Prasad Y, Venkata Ramana Y, Kabir Ahmed S, Murthy KJR : Study on lung function tests and prediction equations in Indian male children. Indian Pediatrics. 40(8): 705-711, 2003.

20. Sudip Ghosh, Anil K. Singh, Battu Aruna, Sangita Mukhopadhyay, Nasreen A. Ehtesham : The genomic organization of mouse resistin reveals major differences from the human resistin: Functional implications. Gene. 305(1-2):27-34, 2003.

21. Sujatha T, Veena Shatrugna, Vidyasagar P, Nazeema Begum, Padmavathy KS, Chenna Krishna Reddy G, Narasimha Rao GV : Timed activity studies for assessing the energy expenditure of women from an urban slum in South India. Food and Nutrition Bulletin. 24(2):193-199, 2003.

22. Suryanarayana P, Kamala Krishnaswamy, Bhanuprakash Reddy G : Effect of curcumin on galactose-inducedcataractogenesis in rats. Molecular Vision. 9: 223-230, 2003.

23. Uma Nayak M, Vishnu Vardhana Rao M, Shahnaz Vazir, Vijayaraghavan K : Innovative use of cluster analysis to evaluate social marketing strategies to control vitamin A deficiency. Ecol. Fd. Nutr. 42 (1): 9-23, 2003.

24. Vijayapusham T, Menon Krishna Kumari, Raghunatha Rao D, Grace Maria Antony : A qualitative assessment of nutrition knowledge levels and dietary intake of schoolchildren in Hyderabad. Public Health Nutrition. 6(7):683-688, 2003.

B. PAPERS PUBLISHED IN PROCEEDINGS

1. Bhat RV : Food quality and safety hazards in India: the way ahead. 30th Kamlapuri Sabharwal Lecture delivered at Lady Irwin College, Delhi on Dec'5, 2003. PP. 1-32 Delhi, Dept. of Food and Nutrition, Lady Irwin College, University of Delhi, 2003.

2. Bhat RV : Risk assessment of monosodium glutamate: Indian perspective. Proceedings of the symposium on safety and usefulness of glutamate as a flavour enhancer: Current status of the knowledge. Symposium held at Brindavan Gardens, Mysore, India. Symposium, 2003.

3. Bhat RV, Sudershan RV : Application of hazard analysis critical control point as a component of risk management option-case studies. In "Proc. IX Asian Congress of Nutrition: Nutrition goals for Asia-Vision 2020", held on Feb'23-27, 2003 at New Delhi, Nutrition Foundation of India. PP 664-668, 2003.

4. Ghafoorunissa : Polyunsaturated fatty acids in Indian diets and their clinical implications. In "Proc. IX Asian Congress of Nutrition: Nutrition goals for Asia-Vision 2020", held on Feb'23-27, 2003 at New Delhi, Nutrition Foundation of India. PP 464-468, 2003

5. Mallikharjuna Rao K, Hanumantha Rao D, Mathur YN, Radhaiah G, Pralhad Rao N : Socio-demographic and nutritional status of tribal population in three ecological zones of Madhya Pradesh. In "Demography and health profile of the tribals (a study of Madhya Pradesh) ed.by Adak, D.K. etal. New Delhi, Anmol Publications. :PP 217-226, 2003.

6. Sivakumar B : Assessment of vitamin a status. In "Proc. IX Asian Congress of Nutrition: Nutrition goals for Asia-Vision 2020", held on Feb'23-27, 2003 at New Delhi, Nutrition Foundation of India. PP 297-308, 2003.

7. Vijayaraghavan K : Diet and nutritional status during drought in India - time trends. In "Proc. IX Asian Congress of Nutrition: Nutrition goals for Asia-Vision 2020", held on Feb'23-27, 2003 at New Delhi, Nutrition Foundation of India. PP 737-745.

8. Brahmam GNV, Madhavan Nair K, Venkaiah K, Harikumar R and Vijayaraghavan K : Developing strategy for prevention and control of Iron Deficiency Anaemia in Mehaboobnagar district of Andhra Pradesh. In proc. IX Asian Congress of Nutrition: Goals for Asia-Vision for 2020. 23-27th February 2003, New Delhi. PP 100.

9. Shahnaz Vazir : Care behaviours and positive deviance among rural children. IX Asian Congress of Nutrition: Goals for Asia-Vision for 2020. 23rd – 27th February 2003. PP.101.

10. Vishnuvardhan Rao M, Narhari Pandit S.N : Longitudnal multivariate data analysis for recognizing nutrition status through anthropometric measurements in children. IX Asian Congress of Nutrition. 23rd- 27th February 2003.

C. SPECIAL REPORTS

1. Nair KM, Ranganathan S, Acharya JS, Kamala Krishnaswamy, Sivakumar B, NIN, Hyderabad : Iron absorption promoters in fortification of edible salt. Final Technical Report, Hyderabad, National Institute of Nutrition, 1-42PP, 2003.

2. National Nutrition Monitoring Bureau : Prevalence of micronutrient deficiencies. NNMB Technical Report No. 22, Hyderabad, NIN (NNMB), 2003.

3. Sivakumar B, Nair KM, Komilla Pareek, Ravinder P, Kamala Krishnaswamy, NIN, Hyderabad : Studies of fortification of wheat flour with iron and folic acid (IDRC GRANT NO:910313-211). Final Technical Report. Hyderabad, NIN, 1-46PP, 2003.

4. Sivakumar B, Nair KM, Vikas Rao V, National Institute of Nutrition, Hyderabad : Establishment of a National Facility for Dry Blood Spot technology for vitamin A estimation, at National Institute of Nutrition, Hyderabad. Final Technical Report, 1-6PP, 2003.

5. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Vishnuvardhana Rao M, Arlappa N, Gal Reddy CH, Mallikharjuna Rao K, Sharad Kumar, Ravindranath M : Diet and nutrition situation in drought affected areas of Andhra Pradesh, 2003.

6. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Ravindranath M, Vishnuvardhana Rao M, Arlappa N, Gal Reddy CH, Mallikharjuna Rao K, Sharad Kumar : Diet and nutrition situation in drought affected areas of Karnataka, 2003.

7. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Sharad Kumar, Vishnuvardhana Rao M, Arlappa N, Gal Reddy CH, Mallikharjuna Rao K, Ravindranath M : Diet and nutrition situation in drought affected areas of Maharashtra, 2003.

8. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Gal Reddy CH, Vishnuvardhana Rao M, Arlappa N, Mallikharjuna Rao K, Sharad Kumar, Ravindranath M : Diet and nutrition situation in drought affected areas of Orissa, 2003.

9. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Vishnuvardhana Rao M, Arlappa N, Gal Reddy CH, Mallikharjuna Rao K, Sharad Kumar, Ravindranath M : Diet and nutrition situation in drought affected areas of Tamil Nadu, 2003.

10. Vishnuvardhana Rao M, Arlappa N, Mallikharjuna Rao K, Ravindranath M : Diet and nutrition situation in drought affected areas of Chattisgarh, 2003.

11. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Arlappa N, Mallikharjuna Rao K, Vishnuvardhana Rao M, Gal Reddy CH, Sharad Kumar, Ravindranath M : Diet and nutrition situation in drought affected areas of Gujarat, 2003.

12. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Anjali Singh, Vishnuvardhana Rao M, Arlappa N, Gal Reddy CH, Mallikharjuna Rao K, Sharad Kumar, Ravindranath M : Diet and nutrition situation in drought affected areas of Madhya Pradesh, 2003.

13. Vijayaraghavan K, Brahmam GNV, Venkaiah K, Mallikharjuna Rao K, Arlappa N, Vishnuvardhana Rao M, Gal Reddy CH, Sharad Kumar, Ravindranath M : Diet and nutrition situation in drought affected areas of Rajasthan, 2003.

14. Current status of IDD in select districts of different regions of the country, NIN, ICMR, Hyderabad, 2003.

15. Vijayaraghavan K, Hanumantha Rao D, Brahmam G.N.V, Venkaiah K, Laxmaiah A, Hari Kumar R, Vishnuvardhan Rao M, Arlappa N, Gal Reddy Ch, Mallikharjuna Rao K, Sharad Kumar and Ravindranath M: Nutrition profile of community in West Bengal. CINI, National Institute of Nutrition, ICMR, 2003.

16. Vijayaraghavan K, Brahmam G.N.V, Sharad Kumar, Vishnuvardhan Rao M, Arlappa N, Venkaiah K, Laxmaiah A, Hari Kumar R, Gal Reddy Ch, Mallikharjuna Rao K, Ravindranath M: Nutrition profile of community in Uttaranchal – State Level Report, National Institute of Nutrition, ICMR and Institute of Applied Statistics and Development Studies, Lucknow, 2003.

D. POPULAR ARTICLES

1. Amulya Rao: Carbonated drinks. Nutrition 37:26-32, 2003.
2. Indira, Ravindranath: Carrots, tomatoes, papaya. Daily. Nutrition. 37(1): 3-25, 2003.
3. Indira, Ravindranath: Mother's milk-ideal nutrition for babies. Nutrition. 37:3-24, 2003.
4. Indira, Ravindranath: Phytochemicals in plant foods. Nutrition.37(2):19-32, 2003.
5. Jayasudha, Udapi: Adolescent nutrition. Nutrition. 37(2):3-18, 2003
6. Nageswara Rao, R: The fat appeal.. Nutrition. 37(3):25-30, 2003.
7. Parekh, Komila; Sailaja, K : Wonders of microwave cooking. Nutrition, 37(4): 16-32, 2003.
8. Subba Rao, GM: International alliance against hunger. Nutrition. 37(4): 3-15, 2003.
9. Shahnaz Vazir: Determinants of food behaviours and nutrition. ICMR Bulletin, Vol.33, No.8, pp 77-82, August 2003.
10. Laxmaiah A and Vijayaraghavan K: Family level preparedness during drought. Health Action, 2003; 4:13-15.

E. OTHERS

1. Bhat RV, Vasanthi S : Food safety in food security and food trade mycotoxin food safety risk in developing countries. IFPRI 2020 Vision for the food, agriculture and environment. Focus 10, Brief 3 of 17, 1-2, Sept'2003.
2. Dube Anil Kumar : Overweight children: a weighty problem. Health Action. 8-12, 2003.
3. Dube Anil Kumar : Health care public relations - A new thrust area. Health Action. 16(2):10-12, Feb'2003.
4. Laxmaiah A, Vijayaraghavan K : Family-level preparedness during drought. Health Action. 16 (4) :13-15, 2003.
5. Polasa K, Nirmala K : Ginger: its role in xenobiotic metabolism. ICMR Bulletin. 33 (6): 1-6, 2003.
6. Shahnaz Vazir : Determinants of the development of food behaviours and nutrition. ICMR Bulletin . 33(8): 77-82 , Aug'2003.
7. Subba Rao GM: Little soldiers: fighting hunger. Health Action. 16(9): 31-33, 2003.
8. Subba Rao GM : Nutrition: an index of development. Health Action. 16(11):4-7, 2003.
9. Subba Rao GM : Nutrition and national development. Population and Dev. Education News Bulletin. 9 (3):4-6, Sept'2003.
10. Suresh C : Travel health. Health Action. 16(6):33-35, 2003.
11. Vajreswari A : Relevance, role & requirement of fats in infant nutrition. In-Touch. 2-6, Oct-Dec' 2003.
12. Veena Shatrugna: Women, health and nutrition. Perspectives, 3-8, June, 2003.

SCIENTIFIC ADVISORY COMMITTEE

NIN/FDTRC

1. Prof. M.K. Bhan …. *(Chairman)*
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PGIME & R, Chandigarh – 160 012

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PGIMERI, Chandigarh

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Dept. of Pediatrics, KEM Hospital
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All India Institute of Hygiene & Public Health
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Dept. of Community Medicine
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18. Prof. N.K. Ganguly
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19. Dr. Padam Singh
Addtl. Director General
ICMR, New Delhi

20. Dr. N.C. Saxena
Chief, RHN Division
ICMR, New Delhi

21. Dr. Rakesh Mittal
DDG (Sr.Gr)
ICMR, New Delhi

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(Member-Secretary)
Officer-in-Charge
NIN/FDTRC, Hyderabad.

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Former Director – NIN
Emeritus Medical Scientist
NIN, Hyderabad

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Hyderabad

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NIMS
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Heavy Water Board, Mumbai

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Saroornagar, Hyderabad

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