ANNUAL REPORT

2008-2009

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RESEARCH HIGHLIGHTS

1. COMMUNITY STUDIES

1.1 NNMB second tribal repeat surveys: Diet and nutritional status of tribal population in India time trends

It was observed that there was no significant change in the dietary patterns of tribal population in the last three decades. However, consumption of income-elastic foods like pulses, GLVs, Sugar and Jaggery has marginally gone up. Prevalence of severe undernutrition has significantly come down from 19.7% in 1985-87 to 7.9% in 2007-08 among both the rural and tribal communities. However, there was a notable shift from moderate to mild malnutrition indicating betterment of nutritional status. Nutritional status of tribes of MP, Maharashtra and Orissa were poor compared to those in the other states.

1.2 Evaluation of Bio-effect of ultra rice on iron status of beneficiaries of mid day meal (MDM) programme – A study in a primary school of Ranga Reddy District, Andhra Pradesh

It was found that the rice fortified with iron when given in the mid-day meal to the school children for over a year, resulted in a significant improvement in their haemoglobin levels and iron stores. However, it was also observed that the children in control group who did not receive ultra rice but got supervised meal as per the norms of MDM Programme also showed Hb improvements with no change in iron status.

1.3 Nutritional status of <3 year children and infant & young child feeding practices among mothers in Medak, Andhra Pradesh: A situational analysis of Sankalp Programme

Government of Andhra Pradesh in collaboration with UNICEF initiated developmental programme **"Sankalp"** in Medak district of Andhra Pradesh. A situational analysis of infant and young child feeding practices and care giving practices among 805 mothers of <3 years children, indicated a significant association between nutritional status and different socioeconomic & demographic variables such as literacy and occupation of parents and presence of sanitary latrine with underweight and stunting, use of LPG and presence of electricity with underweight and wasting.

1.4 Study on relationship between Body Mass Index (BMI) and percent body fat in urban adults

Studies on relationship between Body Mass Index and percent body fat in urban adults indicated that cut-off levels of BMI to indicate Overweight/Obesity, derived based on 25% body fat among men and 30% among women using ROC (receiver operating characteristic) curves, were found to be similar to the cut-off values for Asian Indians suggested by WHO ($BMI \geq 23 kg/m^2$).

2. MICROBIOLOGY AND IMMUNOLOGY

2.1 Allergenicity evaluation of a Bio-preservative skimmed milk fermentate (SMF)

NDDB in association with National Dairy Research Institute (NDRI) Karnal has developed skimmed Milk Fermentate (SMF-Bact) a Bio Preservative, which can be used to increase

shelf life of milk products. Allergenicity evaluation of SMF suggested that it had no allergenicity potential in the concentration tested.

2.2 Immune status of WNIN mutant obese rats with reference to leptin and obesity

It was observed that there were altered T cell subsets and B cells in both the sexes of the strains of mutant rats studied. However, the splenic proliferative response to mitogen decreased in male rats of one strain.

3. BASIC STUDIES

3.1 Isolation and characterization of human milk factor that enhances iron absorption: An exploratory study

In yet another study, enzyme Ferric Reductase activity has been demonstrated in human milk fraction which explains the reason why iron is better absorbed through human milk.

3.2 Studies on mechanism of cytoprotective effect of zinc in Caco-2 cell intestinal model

Results indicated that Zinc inhibits oxygen induced iron uptake and signaling and thus elicits its cytoprotective effects. This also explains the inhibitive effect of zinc on iron absorption.

3.4 Metabolic programming of insulin resistance: Role of maternal and peri/postnatal chromium status in the offspring – Adiposity, glucose and lipid metabolism

Chronic maternal chromium deficiency increased body fat, especially central adiposity in offspring. It altered adipocyte cytokine levels in circulation. It altered lipid metabolism with increased circulating triglycerides and free fatty acid levels. However, it did not alter gene expression. It caused impaired glucose tolerance and increased insulin secretion. Rehabilitation could partially correct these changes.

3.5 Health beneficial effects of plant foods commonly consumed in India: nuts and oil seeds

Very strong correlation was observed between the phenolic content and FRAP and DPPH scavenging activities indicate that phenolics were significant contributors to the anti-oxidant activity of nuts and oilseeds commonly consumed in India.

3.6 Importance of α-crystallin heteropolymer in the eye lens: Oligomeric size, polydispersity and stability

Chaperone-like activity (CLA) of the small heat shock protein α-crystallin is essential for the maintenance of eye lens transparency. The eye lens α-crystallin is a heteropolymer, composed of two homologous subunits, αA and αB. In most vertebrates the ratio of A to B in heteropolymer is 3:1. However, the physiological significance of 3:1 heteropolymer is not known. The current studies have shown that under normal conditions αB-homopolymer exhibits higher CLA than A-homopolymer. In contrast, under stress conditions 3:1 heteropolymer displayed greater CLA. It was further demonstrated that αB-crystallin homopolymer is not only less stable and contributes to light scattering due to aggregation, but it could also involved in coaggregation of other lens proteins in the absence of αA-crystallin. Thus, existence of A and B in 3:1 ratio in lens might have evolved as an advantageous combination to preserve eye lens transparency under diverse conditions to prevent cataract.

3.7 Expression of α-crystallins under hyperglycemic conditions: Role of oxidative stress , transcription factors and dietary anti-oxidants

Expression of small heat shock proteins, αA- and αB-crystallins has been shown to be elevated under various stress and pathological conditions. Diabetes is known to be associated with various metabolic stresses including oxidative stress. For the first time, it has been reported that hyperglycemia induced stress leads to increased expression of αB-crystallin in lens, heart, muscle and brain and αA in retina. Further, it was shown that transcription factor HSF1 could be responsible for up regulation of α-crystallins under hyperglycemic conditions. While increased oxidative stress appears to be a major stimulus for the enhanced expression of αB-crystallin in tissues of diabetic rats, feeding of a dietary antioxidant (curcumin) to diabetic rats attenuated the enhanced expression of αB-crystallin.

3.8 Characterization and significance of a novel fatty acid elongase, ELOVL4, of the eye

ELOVL4 (Elongation of Very Long Chain Fatty Acid 4) is a novel member of human fatty acid elongases whose functional role is currently not known. ELOVL4 gene is expressed in the photoreceptor cells of the retina in a number of species and a mutation (5-bp deletion) in ELOVL4 gene can cause a particular form of macular degeneration. The current studies have shown that expression of this novel elongase is higher in retina compared to lens in many vertebrate species. A comparison of fatty acid profile between lens and retina of given species has enabled us to cling on to the elongation reaction of ELOVL4 that it might be involved in the elongation of fatty acids with a chain length greater than C28. Studies also indicated that expression of ELOVL4 in retina is positively modulated by dietary long-chain polyunsaturated fatty acids, which in turn is associated with the maintenance of integrity of retinal morphology.

4. EXTENSION AND TRAINING

4.1 A study on approaches to nutrition communication

Case studies on approaches taken by various organizations for nutrition communication in different sectors (Government, NGO and R&D) indicated that Nutrition communication activities lack proper planning, monitoring and evaluation components. This makes it difficult to attribute any change in behaviour to a particular communication process.

The selection of specific communication approaches is not primarily based on normative value of the approach but purely based on institutional factors and expectations including organisation's goals, bureaucratic dynamics and budgetary constraints

4.2 A study on coverage of nutrition related topics in print media

The study revealed that the vernacular (Telugu) dailies covered more number of nutrition related articles than the English newspapers and most of these were more on conventional foods rich in nutrients. English dailies published more articles on lifestyle foods like chocolates, beverages, ice-creams etc.

5. FOOD AND DRUG TOXICOLOGY

5.1 Microbiological risk assessment of street foods with special reference to poultry products

The study indicated that 50-70% of samples of poultry foods were found to be contaminated with disease carrying bacteria like Bacillus cereus and Staphylococcus aureus. The vegetable salads were found to be contaminated with Salmonella due to improper handling.

5.2 In vitro chelating potential of thiamine with lead

Earlier studies indicated that thiamine can chelate and thus reduce the uptake of lead in intestines. This was confirmed using in vitro human intestinal cell lines. Therefore correcting thiamine deficiency itself would reduce the risk of lead toxicity in populations at risk.

6. PRE-CLINICAL TOXICOLOGICAL STUDIES

6.1 Safety evaluation of AB-FN-02 having potential anti-osteoarthritic activity

Safety evaluation of a polyherbal drug, AB-FN-02 having potential anti-osteoarthritic activity indicated that it was non-toxic when administered in traditional method with milk and was found toxic when administered as a drug otherwise.

7. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

The first phase of the Indo-US project to find out the nature of mutation in the obese rats developed in the center was completed, with the crossing of WNIN/Ob rats with that of Fischer 344 rats and the preparation of DNA samples from Fo, F1 parents and F2 progenies.

8. OTHERS

Total number of publications by scientists in national and international journals was over 40 with an average impact factor of 2.5.

I. COMMUNITY STUDIES

1 NNMB SECOND TRIBAL REPEAT SURVEYS: DIET AND NUTRITIONAL STATUS OF TRIBAL POPULATION IN INDIA AND TIME TRENDS

The National Nutrition Monitoring Bureau (NNMB) since its inception in 1972 under the Indian Council of Medical Research (ICMR) in the States of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa, West Bengal and Uttar Pradesh has been carrying out diet and nutrition surveys on a regular basis and the results are being published as technical reports. In order to study the time trends in the food and nutrient intake patterns and the nutritional status of individuals in tribal areas, the Bureau carries out repeat surveys once in 5 years by visiting the same set of villages. In case of tribal areas, the baseline survey was carried out during 1985-87, first repeat survey was carried out during the year 1998-99 and the second repeat survey i.e. the present survey was carried out during 2007-08.

GENERAL OBJECTIVE

To assess the current diet and nutritional status of tribal population living in ITDA areas in all 10 NNMB States and to study the time trends. In addition, it is also proposed to assess the prevalence of obesity and hypertension among adult men and women of 20 years of tribal population, as was done in the latest rural surveys.

SPECIFIC OBJECTIVES

1. To assess the food and nutrient intake among different age/ sex/ physiological groups.
2. To assess the nutritional status of individuals in terms of Anthropometry, clinical examination for nutritional deficiency signs.
3. To assess the history of morbidity for previous fortnight among all the individuals covered for anthropometry.
4. To assess the time trends, if any, in the diet and nutritional status of tribal population.
5. To assess the prevalence of obesity and hypertension among the adult men and women (20 years), and
6. To assess awareness about hypertension among adults (20 years) of the tribal community.

COVERAGE

The National Nutrition Monitoring Bureau (NNMB) has carried out second repeat survey in ITDA areas during 2007-08 in 75% of the villages (90) which were already surveyed during 1985-87 and 1998-99 and 25% of the new villages (30), to asses the current diet and nutritional status and time trends among 95,590 individuals and food consumption pattern (34,544) and assessed the prevalence of hypertension among adult men and women (20 years) from 851 villages. Due to logistic problems, the survey could not be completed in the States of Uttar Pradesh and West Bengal.

The following are the salient observations:

- The mean intake of most of the foodstuffs was below the RDI and there was no significant change observed over a period of time
- The consumption of income elastic foods such as green leafy vegetables, milk and milk products, fruits, sugar and jaggery increased marginally.
- The extent of deficit was more for vitamin A, iron, calcium and folic acid among children and pregnant women.
- About 30% of the preschool and school age children had adequate intakes of both protein and calories.

- The overall prevalence of undernutrition (<Median – 2SD) in the form of underweight, stunting and wasting among infants was 36%, 36% and 22% respectively, while it was higher among preschool children (66%, 58% and 22% respectively).
- The time trends indicated that there was significant reduction in the prevalence of severe degree undernutrition (<60% of weight for age of NCHS standards) in preschool children (19.7% to 7.9%).
- The prevalence of chronic energy deficiency was about 40% and 49% among men and women respectively.
- There was also reduction in nutritional deficiency signs like kwashiorkor, marasmus, vitamin A and B-complex deficiencies among preschool children.
- The prevalence of hypertension (SBP 140 mm of Hg and /or DBP 90 mm of Hg) was about 25% among men, while it was 23% among women. About 41% of men and 33% of women were aware of hypertension. About 35% men and 29% women were aware of diabetes mellitus.

Therefore, there is an urgent need to sensitize the community regarding the causes and consequences of undernutrition and obesity, HTN and DM and to educate them about the need for adopting appropriate life styles and dietary habits.

2 EVALUATION OF BIO-EFFECT OF ULTRA RICE ON IRON STATUS OF BENEFICIARIES OF MID DAY MEAL (MDM) PROGRAMME – A STUDY IN A PRIMARY SCHOOL OF RANGA REDDY DISTRICT OF ANDHRA PRADESH

Iron Deficiency Anaemia (IDA) is a major micronutrient deficiency disorder of public health significance affecting millions of women and children in India. Surveys carried out by National Nutrition Monitoring Bureau (NNMB) in 8 States viz., Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Madhya Pradesh, Orissa and West Bengal have shown that the prevalence of IDA is about 70 to 80% among different age, sex and physiological groups.

The major aetiological factor of IDA is dietary inadequacy of iron. The NNMB studies have shown that in about 60% of the rural households the average intake of dietary iron is less than 50% of recommended levels. The Presence of substances such as phytates and tannins in high amounts that interfere with iron absorption in the Indian dietaries, and lack of absorption promoters such as vitamin C, further compound the problem.

The PATH-USA has developed technology of fortification of rice using Ultra Rice (UR), a premix of extruded rice flour having iron as iron pyro-phosphate and proposes to transfer the technology to developing countries, including India. However, the bio-effect of this technology in improving iron status has not been proved in the Indian context. It was hypothesized that consumption of rice fortified with iron UR by 5-11 year old school children, at a level to bridge the deficit in the dietary intake of iron, through MDM over a period of 9 months will have positive impact on iron status of the beneficiaries.

During the year 2006-07, a placebo controlled randomized double blind feeding trial was carried out by National Institute of Nutrition, Hyderabad, to assess the impact of consumption of rice fortified with iron UR through Mid Day Meal (MDM) on haemoglobin and ferritin status of beneficiaries of the programme in a primary school in *Ranga*

Reddy district of Andhra Pradesh. The study revealed that i) the rice fortified with iron UR is acceptable among the MDM beneficiaries, ii) there was no loss of iron due to pre-cooking and cooking processes, iii) feeding trial was operationally feasible, iv) an effective mean feeding days of 76 per child was achieved, which was inadequate to demonstrate an impact conclusively. Based on these results, it was decided to conduct the impact evaluation afresh during the academic year of 2007-08, for an effective duration of 9 months, by adopting similar protocol in the same school.

HYPOTHESIS

Consumption of rice fortified with iron UR by 5-11 year school children, at a level to bridge the deficit in the dietary intake of iron, through MDM over a period of 9 months will have positive impact on iron status of the beneficiaries.

OBJECTIVE

To assess the impact of consumption of rice fortified with iron UR on iron status of beneficiaries of the MDM programme, over a period of nine months.

STUDY DESIGN

The study was a placebo controlled randomized double blind feeding trial carried out among children in the age group of 5+ to 11+ years in a primary school, where MDM programme is being implemented.

SAMPLE SIZE

A sample size of 70 children per group was calculated, assuming an expected overall mean increment of 0.56 g/dL in haemoglobin with an SD of 1.04 g/dL (based on the results of the previous study), 95% confidence interval, 80% power of estimate, and 20% of attrition.

SAMPLING PROCEDURES

The current study has been carried out in the same government primary school in *Keesara* village of *Keesara* Mandal, Ranga Reddy district, Andhra Pradesh, where the earlier study was implemented during the academic year 2006-07. Necessary permission for carrying out the study was obtained from the Department of District Education, Andhra Pradesh during July, 2007.

The selected school had 230 children, consisting of 83 boys and 145 girls of 5+ to 11+ years age group. Of the 230 children, 70 were newly admitted to the school during the current academic year of 2007-08, while the rest were old students.

Inclusion Criteria

Apparently healthy children in the age group of 5+ to 11+ years participating regularly in the MDM programme, having haemoglobin levels of ?7 g/dL, those with weight for age, height for age and weight for height ?median – 3 SD of NCHS reference values were considered for inclusion in the study (n=146).

Exclusion Criteria

Children who were severely anaemic with haemoglobin levels of <7 g/dL (n=4), undernourished (weight for age, height for age and/or weight for height < Median – 3SD of the NCHS reference values) (n=3), not participating in MDM (n=10) or participating irregularly in MDM (n= 65) were excluded from the study.

BASE LINE SURVEY

Institutional Diet Survey

Institutional diet survey by weighment method was carried out on two days with different menu (Tamarind rice, and rice with *sambar* and egg). Average consumption of MDM by the children was assessed in both the groups. In addition, individual intake of MDM was assessed among a sub-sample of 24 randomly selected children (12 in each group) belonging to different age/gender strata from both the groups.

ANTHROPOMETRY

The heights and weights of the children were measured by using anthropometer rod

and Seca electronic balance, by adopting standard methodology.

BLOOD CHEMISTRY

Two millilitres of blood samples were drawn from ante-cubital vein of selected children for estimation of haemoglobin by cyanmethae-moglobin method, serum ferritin by an in-house ELISA method and C-reactive protein by a commercial ELISA kit (Abazyme, LLC, MA, USA).

INTERVENTION

Intervention was carried out from the month of August 2007 to April 2008. The children of both the groups received the respective coded rice along with common regular side dishes on all school working days (excluding Sundays and holidays). Care was taken to cook and serve the fortified and unfortified rice separately and ensured that both the groups received their respective coded rice. The entire process was monitored by field staff under the supervision of research investigators from NIN.

MORBIDITY AND SIDE EFFECTS

Morbidity and side effects, if any, were assessed and recorded every day and compiled once in every 15 days.

END LINE SURVEY

At end line, i.e. in the 2nd week of April, the anthropometric measurements of children were taken, institutional diet survey was carried out and blood samples were collected from the children for estimation of haemo-globin, serum ferritin and C-reactive protein.

Data analysis & statistical models used

Data was analyzed using SPSS version 15.0 for Windows to assess the impact of intervention on iron status of children. Descriptive statistics were computed to analyze the central tendencies and dispersions. Paired t-test and Wilcoxon Signed Ranks test were carried out to see the difference between baseline and end line for each group of haemoglobin and ferritin. Student 't' test was carried out and 95% confidence intervals of increments in haemoglobin and ferritin were computed to study the efficacy of intervention. In addition, following three types of linear regression analysis were performed to evaluate the effect of intervention on haemoglobin and ferritin levels.

RESULTS

At the end of the analysis decoding was done by the Director, NIN. It was observed that the group 'B' was control and 'Y' was experimental Group. The results of the analysis are given below:

1. The analysis revealed that the iron content of ultra rice premix was 10.9 ± 0.1 mg/g on dry weight basis and was same as that used in the preliminary study.
2. It was observed that the mean iron content was about 2 mg in unfortified and 20 mg in fortified rice. Iron content of fortified and unfortified rice showed minimal variation during the study period.
3. The mean age of boys and girls was similar in both the control and experimental groups. There was a mean increment of about 4 cm in height and about 3 kg in weight in both the groups.
4. Though not statistically significant, there was a decline in the prevalence of underweight while stunting and wasting remained similar in control and experimental groups.
5. The mean Hb and serum ferritin levels among children were comparable between the two study groups at base line. A significant ($p<0.001$) increase in mean Hb levels was observed in both the groups at end line. However, the extent of change was not significantly different between the two groups.
6. The mean serum ferritin levels increased significantly ($p<0.001$) from 24.7 to 32.9

µg/L in the experimental group after intervention. In control group the levels decreased marginally.

7. The mean CRP levels remained similar between and with in groups both at baseline and end line of the study.
8. The total number of child feeding days achieved was 8667 in the experimental and 8632 in the control groups. The mean number of child feeding days achieved was 133 per child in the control group and 138 per child in the experimental group, indicating compliance rate of about 80%.
9. The average intake of rice ranged from about 103 to 111 g per child per meal and was comparable among the study groups both at base line and end line. The average intake of iron ranged from 1.9 to 2.1 mg per child per meal in control group and experimental group.
10. There was a mean increment of about 1g/dL in control and experimental group. No significant difference in the increments of haemoglobin was observed between groups.
11. The mean change in the ferritin levels was significantly ($p<0.001$) higher in the experimental group (8.2 µg/L) compared to the control group (-3.0 µg/L), with 95% CI of 5.9 – 16.5 µg/L. Similar results were made even after excluding the data of children with C reactive Protein levels of ? 0.8 µg/L (indicative of infection).
12. The change in Haemoglobin and ferritin values between base line and end line was assessed by three methods of regression analysis. All the three models viz. absolute changes, analysis of covariance and Residual change yielded similar results for haemoglobin as well as ferritin. The change was statistically significant with respect to ferritin ($p<0.001$).
13. The proportion of children falling sick reduced significantly ($p<0.001$) at end line in both the groups with the extent of decrease being significantly higher ($p<0.01$) in the experimental group compared to the control group.

CONCLUSIONS

The findings of the study indicate that rice fortified with iron UR improves the iron stores, reduces the morbidities among school children participating in the mid-day meal programme suggesting that fortification of rice with iron ultra rice through mid day meal programme can be considered as a strategy to prevent iron deficiency among children.

3 ASSESSMENT OF NUTRITIONAL STATUS OF <3 YEARS CHILDREN AND INFANT AND YOUNG CHILD FEEDING AND CARING PRACTICES IN MEDAK DISTRICT OF ANDHRA PRADESH

Government of Andhra Pradesh, with UNICEF assistance, 'SANKALP' is being implemented in the district of Medak. The objective of the programme was to reduce undernutrition and morbidity among <3 years children by improving quality of care at Anganwadi centres, making the centers more child attractive and empowerment of parents of <3 year children and community. The programme is already being implemented in various blocks in the district at various levels.

Therefore, a situational analysis was carried out to know the current status of undernutrition and Infant and Young Child Feeding (IYCF) Practices.

GENERAL OBJECTIVES

To assess the current nutritional status of <3 year children, infant and young child feeding practices among women, extent of coverage of beneficiaries under antenatal care, immunization, supplementary feeding, micro-nutrient supplementation Programmes etc. in Medak district of Andhra Pradesh.

SPECIFIC OBJECTIVES

The specific objectives of the study are to assess:

1. Extent of antenatal care (ANC),
2. Prevailing infant and young Child feeding practices among mothers
3. Extent of recording of birth weight and growth monitoring,
4. Nutritional status of <3 year children,
5. Incidence of morbidities such as fever, diarrhoea, dysentery & acute respiratory infections among <3 year children,
6. Participation of the target beneficiaries in supplementary feeding, immunization and supplementary nutrition programmes,
7. Personal hygiene, psycho-social care behaviours of mothers, and knowledge and practices of service providers.

METHODOLOGY

It was a cross sectional community based study carried out by adopting systematic random sampling procedure, 40 Anganwadi center villages were covered for various investigations in the district by proposition of Anganwadi centre in each block. About 20 children under 3 years were covered from each AWCs. The data on various parameters such as ante-natal care (ANC) during pregnancy, breast feeding and caring practices, anthropo-metrical measurements, immunization etc were collected.

The following are the salient observations:

- A total of 805 mothers and children of <3 years were covered for this survey. Of which, 49% were boys and 51% were girls.
- About one third of HHs (33%) belonged to either schedule caste (25%) or schedules tribes (8%).
- About 60% were nuclear and extended nuclear families and 41% of fathers and 60% of mothers of index children were illiterate.
- About 30% of HHs did not possess any agricultural land and were engaged in either agricultural or other labour (50%). About 30% of the mothers of index children were housewives. A large proportion of HHs (79%) lived in semi pucca houses.
- About half of the HHs (49%) was using free flowing salt and 60% were using adequately iodized salt.
- Majority (96%) of pregnant women undergone ANC services of which about 89% had $\geq$3 ante natal check-ups. About 70% of deliveries took place either in govt. or private hospitals and majority (65%) was conducted by a medical doctor.
- About 85% mothers feed colostrum to the new born. About 22% of mothers each initiated breast feeding within 1 hour and 1-3 hours of delivery and about 36% initiated after 24 hours of delivery.
- Pre-lacteal feeds such as glucose water, honey etc was given to 45% of infants. Only 41% children received exclusive breast-feeding for 6 months.
- About 91% of children were completely immunized. About 49% of children received one dose and 31% received two doses of massive vitamin A during the previous one-year. The prevalence of underweight, stunting and wasting was 38.6%, 29.6% 22.3% respectively according to new WHO standards.
- Literacy of parents, occupation of parents and presence of sanitary latrine were

found to be significantly associated with under weight and stunting ($p<0.01$).

- About 50-80% of AWWs were aware of all the objectives of ICDS, about 73% villages had trained birth attendant. Anganwadi workers were present in about 88% villages, while LHV in 33% and ANM in 15% of villages.

RECOMMENDATIONS

Improving the knowledge of AWWs about child care, regular growth monitoring, improved immunization services, promoting breast-feeding and timely complementary feeding as well as improvement in socioeconomic and literacy of parents will help in improving the nutritional status of children.

4 STUDY ON RELATIONSHIP BETWEEN BODY MASS INDEX AND PERCENT BODY FAT IN URBAN ADULT POPULATION

Obesity is a condition of excessive fat accumulation in the body to the extent that health and well being are adversely affected. Inview of its convenience and high specificity in detecting subjects with a high percentage of body fat, body mass index (BMI) has been frequently used as an indicator of relative fatness and classification of obesity. Studies have documented that relationship between BMI and percent body fat varies with age, gender and ethnicity. Moreover, Asian Indians have had higher morbidity at lower BMI values than Caucasians. The criteria currently being used to classify overweight or obesity is BMI and waist circumference in adult Europeans, but the same cut-off levels may not be appropriate for Asian Indians.

BMI has a limitation that it can not distinguish between fat mass and fat free mass. This limitation may become important issue when comparing ethnic groups with distinctively different body proportions or physique. The BMI is unable to distinguish into Fat Free Mass Index (FFMI) and Fat Mass Index (FMI). The potential advantage is that only one component of body weight i.e., FFM or FM is related to height squared. These indices are not yet wide applications, probably because appropriate reference standards are yet to be established.

In the present study an attempt was made to study the relationship between body mass index (BMI) and percent body fat (PBF) and also to assess the levels of FFMI and FMI by age and gender in urban adult population.

GENERAL OBJECTIVE

To study the relationship between body mass index and percent body fat in an urban adult population of Hyderabad.

SPECIFIC OBJECTIVES

1. To assess the body mass index and per cent body fat among 20-60 years adults of different income groups.
2. To study the relationship between the BMI levels and percent body fat among individuals by income group.
3. To assess the levels of Fat Free Mass Index and Fat Mass Index by age and gender.
4. To assess the fasting blood sugar levels and lipid profile in a sub sample of subjects selected for the study.

METHODS

Study Design

It is a community based cross sectional study among adult men and women of 20-60 years age group in different income groups.

Computation of sample size

Assumed a correlation (r) of 0.7 between BMI and percent body fat, at 5% level of significance, with 80% of power, the required

sample size arrived at was 14. Hence, 14 individuals in each BMI unit (from BMI of <20 to 30 kg/m^2), a total of 168 individuals for each gender are required. The study was carried out in various socio-economic groups in the city of Hyderabad.

INVESTIGATIONS

Information on socio-economic and demographic particulars such as community, family type, size & income, literacy status of the selected subjects and their history of morbidity, such as diabetes, hypertension and CHD was also collected. Anthropometric measurements including height, weight, fat folds at triceps, biceps, sub-scapular, supra-iliac, waist and hip circumference were taken on all the selected subjects. The sum of all the skin folds were used for calculating body density using standard equation by Durnin and womersely (1974) and percent body fat was calculated using Siri's equation (1956). The fat mass and fat free mass indices are equivalent concepts to the BMI.

The salient observations are as follows:

A total of 1032 individuals i.e., 278 from HIG, 365 from MIG and 389 from LIG were covered for the present study including both men and women, spread over different BMI units (<20 kg/m^2 to ?30 kg/m^2). The mean annual family income was Rs 595914, 221636, and 96936 among men of HIG, MIG and LIG respectively. Similarly the mean annual income was Rs.750608, 205327 and 95004 among women of HIG, MIG and LIG respectively. Among men, the prevalence of hypertension (SBP≥ 140 and/or DBP ≥ 90mm Hg) was about 22%, 10% and 16% among HIG, MIG and LIG respectively, while it was 16%, 11% and 10% among women respectively.

- The diabetes (FBS ? 126mg/dL) was reported by 16.2%, 13.6% and 5.5% of men in HIG, MIG and LIG respectively, while it was 7.5%, 7.5% and 5.5% respectively among women.
- About 3% of men in HIG, 1.3% in MIG and nil in LIG reported CHD, while it was 0.4% among women of LIG.
- A significant correlation was observed between BMI and percent body fat, waist circumference, waist to Hip ratio among both men and women of different socioeconomic groups.
- No significant differences were observed in the mean fasting blood glucose levels, serum triglycerides and total cholesterol in different income groups.
- The appropriate cut off levels of BMI (Men: 23kg/m^2, sensitivity: 86.9%; specificity: 69.7% and women: 22kg/m^2, sensitivity: 88%; specificity: 90%) to indicate overweight, derived based on 25% body fat among men and 30% among women by using ROC analysis, which are similar to that suggested by WHO cut off values for Asian Indians (BMI: >23kg/m^2).
- Similarly, the waist circumference cut off levels was also derived as 85cm for men and 71cm for women when all the income groups were pooled, which are marginally even below that of WHO cut off levels suggested for Asian Indians (men: 90cm and women: 80cm).
- The mean values of Fat Mass Index (FMI) are increased with increase in age. The median fat mass index was 4.4 among men of 20-30years, followed by 6.7 in 30-40-30years, 7.4 in 40-50years and 8.4 in 50-60years. Females had higher fat mass than males and is ranged from 6.5 in 20+ yrs to 10.6 in 50+ yrs.
- The median Fat Free Mass Index (FFMI) is 17.6 in 20-30years, 19.2 in 30-40, 17.9 in 40-50years and 17.3 in 50-60 years. Similar trend was also observed among women.
- According to ROC analysis, the FMI value at 25% body fat is 6.3 kg/m^2 (Sensitivity 88% specificity 93%) among men and at 30% body fat it is 6.64 kg/m^2 (sensitivity 95% specificity 97%) among women.

The fat mass index at different BMI values were relatively higher compared to healthy Caucasian adults.

5 ENDEMIC KIDNEY DISEASE IN THE VILLAGES LOCATED IN THE MICA BELT OF NELLORE DISTRICT, ANDHRA PRADESH- A PILOT STUDY

The global End Stage Renal Disease (ESRD) patient population continues to grow at an alarming rate due to a number of factors. The etiology of chronic kidney disease sited in Indian studies was chronic interstitial nephritis apart from diabetes & hypertension. Chronic interstitial nephritis is presumed to be due to drugs or environmental toxins, and the possible environmental toxins include water and food related toxins. Several studies have shown that there was a strong association between Silica and kidney disease. Inhalation of silica dust during the mining process lead to the development of nephropathy.

Experimental studies on animals have shown that high levels of Silica in drinking water cause kidney disease.

Therefore, it is proposed to carry out a pilot study to assess the approximate prevalence of kidney disease, which will be useful in calculation of sample size for the proposed comprehensive study in mica belt of Nellore district.

RATIONALE OF THE STUDY

In response to print and electronic media reports of 4 deaths and large number of people suffering from kidney diseases at Uchapally village, located in mica belt of Nellore district, National Institute of Nutrition (NIN) and Nizam's Institute of Medical Sciences (NIMS), Hyderabad carried out a rapid exploratory cross-sectional survey to know the extent of the kidney disease in Mica belt of Nellore district. The rapid assessment study revealed that, about 92% the study subjects (48 out of 52 subjects) were in different stages of kidney disease. When projected to the total population of the village, the prevalence of kidney disease was 10%.

On the basis of the results a detailed study proposal was presented before the NIN scientific advisory committee during 2008.

The SAC members recommended to carry out a pilot study in a four villages to arrive at the prevalence of kidney disease, which would be utilized to compute sample size for the comprehensive study proposal.

The pilot study was carried out in four (2 village where mica mine are located and 2 villages in mica area, where the ground water was the source of drinking water) villages located in mica belt.

OBJECTIVE

To study the prevalence of kidney disease based on estimation of Glomerular Filtration Rate (GFR) among the adult population from the selected villages located in the mica belt.

INVESTIGATIONS

1. Household Demographic and Socio-economic particulars.
2. Clinical examination, including measure-ment of BP
3. Estimation of GFR from serum creatinine using the MDRD Study equation. This equation uses serum creatinine in combination with age, sex and race to estimate GFR.

RESULTS

A total of 493 (Men: 234 & Women: 259) adult subjects were covered for the survey. The mean age is 46 years (18-82 years). The major occupation either agricultural (41.4) or non-agricultural (20.9%) labour, while in 14% of households, agriculture was the major occupation. About 36% of the subjects were working in the mica mines, where the mines are located in and around the villages, while 8% in villages are without mica mines. The main source of drinking water is bore well (86%) where mica mines are absent, while

open well (66%) is the major source in the villages where mines are present.

In general, the history of consumption of NSAIDS (Pain killers) was reported by 26% of subjects, while its consumption was reported higher (37%) in the villages where the main source of drinking water is bore well. About 14% and 4% of subjects reported that they were suffering from hypertension and diabetes, respectively.

However, on recording the blood pressure, the prevalence of hypertension was 20%, and significant differences were reported between both types of villages.

The percent of subjects suffering from breathlessness was 28% where the mines are located in and around the village, while this proportion was 12% in villages without mica mines. Similarly, about 28% of subjects reported that they had back pain/knee pain/ankle pain.

In about 18% (87) of subjects the serum creatinine was reported high (>1.5 mg/dl). The proportion of subjects with proteinuria was 21%, while the urinary sugar was positive in about 6% of subjects. In general, the prevalence of kidney disease was about 34%.

II. MICROBIOLOGY & IMMUNOLOGY

1 ALLERGENICITY EVALUATION OF A BIO-PRESERVATIVE SKIMMED MILK FERMENTATE (SMF)

The Dairy products like *khoa, paneer* are popularly known and consumed by all segments of population in India. Therefore supply of quality product having a longer shelf life is of prime concern. To ensure longer shelf life of these Food/Dairy products, NDDB in association with National Dairy Research Institute (NDRI), Karnal has developed skimmed Milk Fermentate (SMF-Bact) based bio-preservative with an intention to promote it as a bio-preservative.

In view of using this product as a food additive for human consumption, the safety profile of such a product is of primary concern. The safety evaluation is also required as per the Prevention of Food Adulteration Act 1954, (Food safety and standards Act 2006, when implemented). The Pre – Clinical Safety and allergenicity potential of the SMF as per Schedule Y of Drugs Controller General of India (DCGI) and PFA was done.

METHODS

As part of PCT, the test for Allergenicity was conducted in Balb/C mice (24M + 24F), which were divided into 4 groups after conditioning them for 10 days.The Group-I animals received Phosphate Buffer Saline(PBS), which served as Vehicle control (VC) and Group-II received Ovalbumin (OVA)(100µg/ dose), which served as positive control. Group-III and IV received Skim Mlik Fermentate-Bacteriocin (SMF-Bact) 100µg and 200µg respectively by intra peritoneal route, weekly once for seven weeks. The blood samples were collected on day 0 (Baseline), 14th, 28th, 42nd for determing serum IgE and IgG levels by ELISA. In addition, Passive cutaneous anaphylaxis (PCA) test was conducted to measure IgE response. All the experimental mice were monitored bi-weekly for live phase cage side activity along with other clinical allergenicity profile. At the end of the study all animals were euthanizated and the organs were collected for gross necropsy and histopathology.

To evaluate PCA reaction the sera collected from sensitized animals (groups-I, II, III and IV) were administered intra dermally (ID) to naive mice. Six hours later Wheal and Flare reaction were studied at the ID site after Intra venous injection of SMF- Bact or OVA or PBS along with Evans blue. Wheal and Flare reaction was observed at the site of injection in naïve animals that received sera of animals exposed to OVA (group-II). Where as, no such reaction was observed in animals that received sera of animals exposed to test material. There were no Gross necropsy changes.

RESULTS

1. There were no preterminal deaths and no abnormal clinical signs and symptoms were reported in the animals exposed to test material till the end of the experiment.
2. Clinical signs of allergenicity that is hair loss, lacrimation, nasal excretion etc. were not observed in the animals that received test material in various concentrations.
3. The serum IgE and IgG levels in test compound group were comparable to Vehicle control (PBS), while animals treated with ovalbumin (OVA-positive control) showed a significantly increased IgE and IgG levels (Figure 2 & 3).
4. Passive cutaneous anaphylaxis (PCA) reaction was positive when serum sample of mice exposed to OVA sensitized animals was used, while there was a negative PCA

Fig 1. Passive Cutaneous Anaphylaxis(PCA)

SMF-BACT sensitized sera (negative PCA)
OVA sensitized sera(positive PCA)

Fig 2.

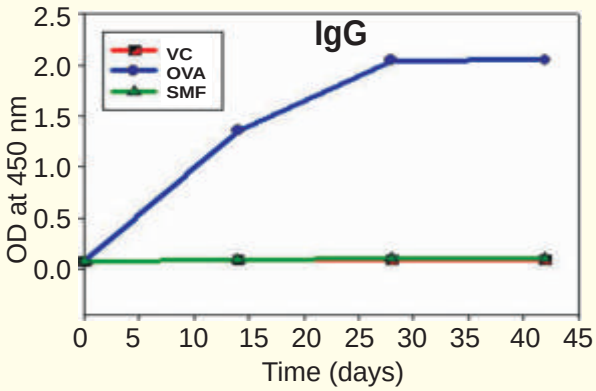
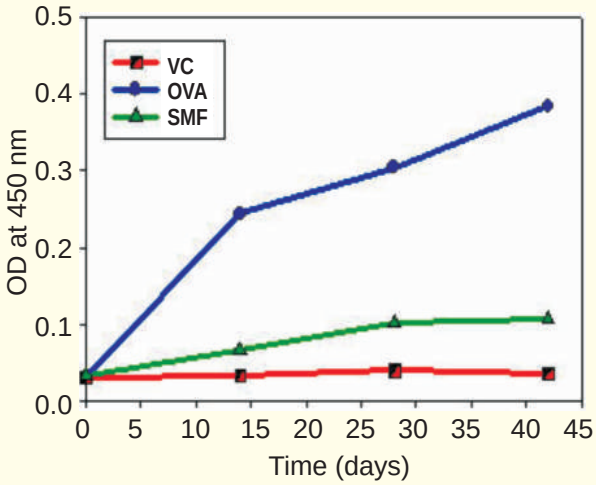


Fig 3



when serum from SMF-Bact sensitized animals was used; suggesting lack of sensitization with SMF-Bact (Figure 1).

CONCLUSION

PCA was negative and IgE response was minimal and was comparable to vehicle control in SMF-Bact received animals, suggesting no allergenicity potential in SMF-Bact with the concentration tested.

2 IMMUNE STATUS OF WNIN MUTANT OBESE RATS WITH REFERENCE TO LEPTIN AND OBESITY (IMMUNE PROFILE OF WNIN/Gr-Ob AND WNIN/Ob MUTANT OBESE MODELS)

Over-nutrition leading to obesity results in coronary heart disease, hypertension and diabetes mellitus. However, clinical and epidemiological data support the evidence that the incidence of infectious diseases is also higher, antibody response to vaccines and wound healing is poor in obese. At NIN, there are two mutant obese models WNIN/Ob and WNIN/Gr-Ob both of which develop kidney dysfunction and tumors as they cross one year of age and their life span is short (1 ½ year vs. 3 years of normal rats) suggesting impaired immune response. Thus in the present investigation the immune profile of these mutant obese models were studied to see whether the immune response is impaired or not.

To study the immune profile three months old obese and lean animals of WNIN/Gr-Ob and WNIN/Ob of both the sexes were taken. The animals were sacrificed and spleen of obese and lean animals was collected under aseptic conditions. The spleen was then processed to perform splenic lymphocyte proliferation assay by using tritiated thymidine incorporation method and T and B cell counts by flow cytometry.

RESULTS

WNIN/Gr-Ob males (Fig 4A & 5A):

The body weight of obese (486±8.33g**)** animals was significantly higher compared to lean (289±8.12g). The spleen weight/g body weight of obese (1.1±0.05 mg) was significantly lower compared to lean (1.9±0.11mg). The range of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells in obese were 18-37% and 12-16% and in lean it was 31-37% and 12-25%. The % of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells in obese were significantly lower than lean. The total $CD3^+$ T cells ranged from 25-41% and 32-50% in obese and lean males respectively and as expected there was a trend towards decreased $CD3^+$ T cells in obese compared to lean. The total B cells ranged from 22-33% in both obese and lean males. However, the total B cells were significantly low compared to lean. The proliferative response of unstimulated cells ranged from 610-1265 and 695-2700 CPM in obese and lean males and was comparable between obese and lean. The proliferative response to mitogen in obese and lean ranged from was 1785-7670 and 4510-30855 CPM and was low in obese compared to lean.

WNIN/Gr-Ob females (Fig 4B & 5B):

Obese (400±3.9g) showed significantly higher body weight compared to lean (209±5.3g). The spleen weight/g body weight of obese (1.3±0.05mg) was lower than lean (2.0±0.07mg). The range of $CD4^+$ helper T cells, total $CD3^+$ T cells and total B cells in obese were 21-37%, 30-43% and 8-26% whereas in lean it was 32-43%, 32-43% and 29-31% respectively. There was a significant reduction in $CD4^+$ helper T cells, total $CD3^+$ T cells and total B cells in obese compared to lean. The $CD8^+$ cytotoxic T cells in obese and lean ranged from 21-37% and 32-43% and were comparable between obese and lean. The proliferative response of unstimulated cells in obese and lean females ranged from 960-3150. The proliferative response to mitogens ranged from 13530-30020 and16625-36740 in obese and lean females respectively. Thus the splenic proliferative response of unstimulated cells and proliferative response to mitogen were comparable between obese and lean.

Fig: 4A

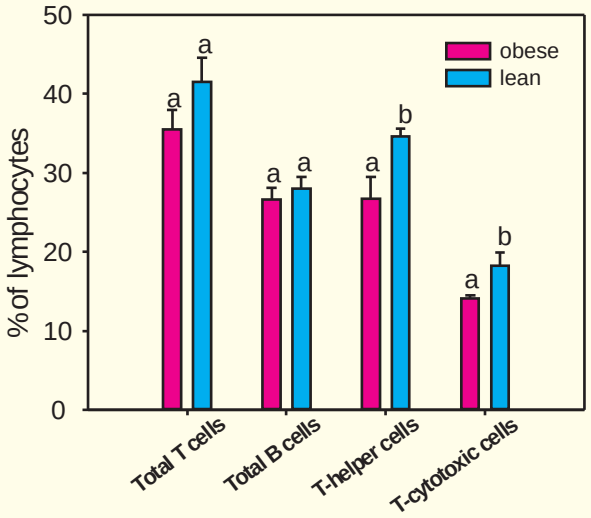


Fig: 4B

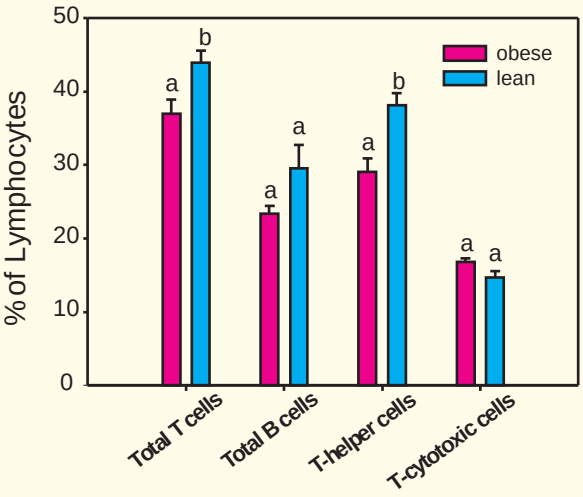


Fig 4: splenic lymphocyte counts were analyzed by flow cytometry in obese and lean of 3 months old WNIN/Gr-Ob and are expressed in terms of percent cells.4A: Males; 4B: females. Values are mean ± S.E ; n=8. Differences between groups were analyzed by a one way analysis of variance at $P<0.05$. Means that don't share common letter are significantly different**.**

Fig: 5A

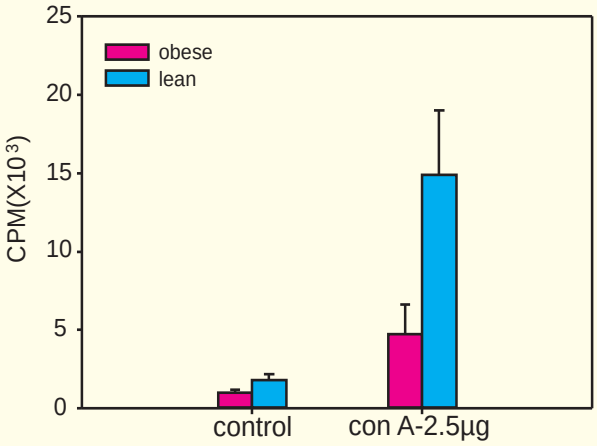


Fig: 5B

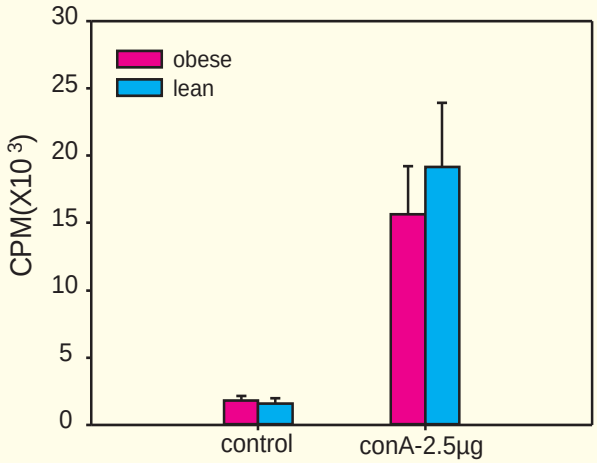


Fig 5: Proliferation of splenic lymphocytes with concavalin A at 2.5µg/1x10^6 cells was studied in obese and lean of 3 months old WNIN/Gr-Ob by incorporation of ^{3}H thymidine and is expressed as counts per minute. 5A: Males; 5B: Females. Values are mean ± S.E; n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share common letter are significantly different.

Across sexes (Fig 4 & 5):

As expected the body weights of obese and lean males were significantly higher than female animals. Except for CD8$^+$ cytotoxic T cells and splenic proliferative responses to mitogen, all other parameters were comparable across sexes in obese. However, obese males had significantly higher CD8$^+$ T cells and lower splenic proliferative response to mitogens compared to females. In lean animals all parameters were comparable across sexes.

WNIN/Ob males (Fig 6A & 7A):

The body weight of obese (558±11.40g) was significantly higher than lean (296± 16.37g). The spleen weight/g body weight of obese (2.2±0.4mg) was comparable to lean (2.6±0.4mg). The % of CD4$^+$ helper T cells and total CD3$^+$ T cells ranged from15-31% and 28-42% in obese whereas in lean it ranged from19-32% and 23-47% . The CD4$^+$ helper T cells, total CD3$^+$ T cells in obese were comparable to that of lean. The range of CD8$^+$ cytotoxic T cells and total B cells in obese was 13-25% and 12-23% whereas in lean it ranged from 9-17% and 15-37%. There was increased CD8$^+$ cytotoxic T cell and a trend towards reduction of total B cells in obese compared to lean. The proliferative response of unstimulated cells ranged from 320-910 and 320-1150 CPM in obese and lean. The proliferative response to mitogen in obese and lean males ranged from 570-18855 and 1145-14490 CPM respectively. The splenic proliferative response of unstimulated cells and proliferative response to mitogens was comparable between obese and lean.

WNIN/Ob females (Fig 6B & 7B):

Obese animals (462±17.44g) had a significantly higher body weight than lean (208±4.44g). The spleen weight/g body weight was lower in obese (1.5±0.01mg) compared to lean (2.4±0.01mg). The range of CD4$^+$ helper T cells and total CD3$^+$ T cells in obese was 20-33% and 29-46% whereas, in lean it was 29-33% and 38-48%. The CD4$^+$ helper T cells and total CD3$^+$ T cells were significantly low in obese compared to lean. The range of CD8$^+$ cytotoxic T cells and total B cells in obese was 12-22% and14-30% whereas in lean it was 11-18% and 14-40%. The CD8$^+$ cytotoxic T cells and total B cells were comparable between obese and lean. The proliferative response of unstimulated cells in obese and lean females

ranged from 490-1055 and 265-840 whereas the proliferative response to mitogen ranged from 830-10655 and 1410-13270 in obese and lean respectively. The splenic proliferative response of unstimulated cells and proliferative response to mitogen in obese were comparable to lean.

Fig: 6A

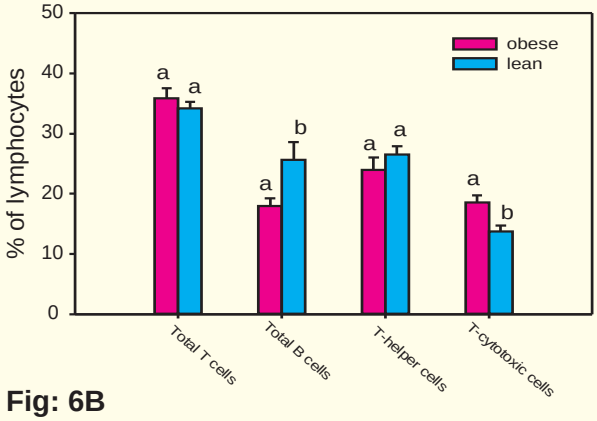


Fig: 6B

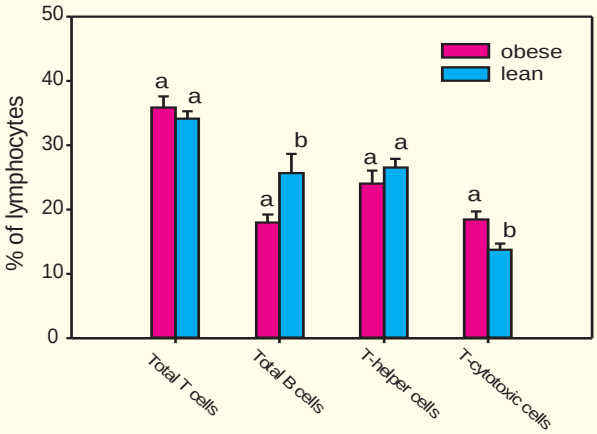


Fig 6: splenic lymphocyte counts were analyzed by flow cytometry in obese and lean of 3 months old WNIN/Ob and are expressed in terms of percent cells. 6A: Males; 6B: females. Values are mean ± S.E ; n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share common letter are significantly different.

Across sexes (Fig 6 & 7)

The body weights of male obese and lean animals were significantly higher than female animals. All the parameters were comparable across the sexes in obese. However, in leans except for $CD4^+$ helper T cells and total $CD3^+$ T cells all other parameters were comparable. The $CD4^+$ helper T cells and total $CD3^+$ T cells were higher in females compared to males.

Fig: 7A

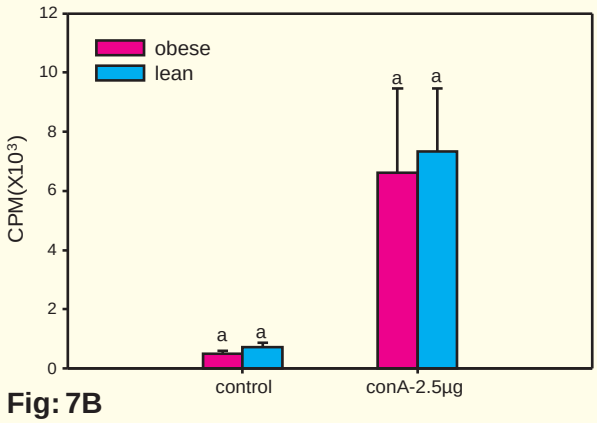


Fig: 7B

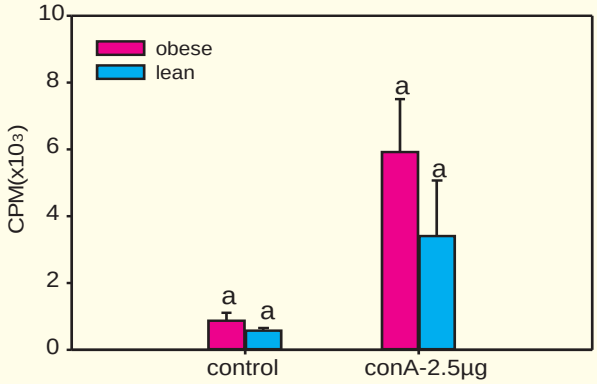


Fig 7: Proliferation of splenic lymphocytes with concavalin A at 2.5µg/1x10^6 cells was studied in obese and lean of 3 months old WNIN/Ob by incorporation of ^{3}H thymidine and is expressed as counts per minute. 7A: Males; 7B: Females. Values are mean ± S.E; n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share common letter are significantly different.

ACROSS STRAINS

Males

Obese: Obese of WNIN/Ob showed significantly higher body weight and spleen weight/g body weight than obese of WNIN/GR-Ob. All parameters except for $CD8^+$ cytotoxic T cells were comparable across strains. The $CD8^+$ cytotoxic T cells were

significantly higher in obese of WNIN/Ob than in obese of WNIN/GR-Ob.

Lean: The body weight and spleen weight/g body weight were comparable across strains. The $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells and total $CD3^+$ T cells were significantly higher in WNIN/Gr-Ob lean compared to the lean of WNIN/Ob.

However, there was no significant difference in the total B cells. Although the splenic proliferative response of unstimulated cells was significantly high in WNIN/Gr-Ob lean compared to WNIN/Ob lean whereas the proliferative response to mitogens was comparable across strains.

Females

Obese: Obese of WNIN/Ob showed statistically significant higher body weight than obese of WNIN/Gr-Ob. Except for splenic proliferative response all other parameters were comparable across the strains. The splenic proliferative response of unstimulated cells and proliferative response to mitogen was significantly higher in WNIN/Gr-Ob obese compared to WNIN/ Ob obese.

Lean: The body weights of lean between the two strains were comparable. The spleen weight/g body weight of WNIN/Ob lean was significantly higher than the lean of WNIN/Gr-Ob. The $CD8^+$ cytotoxic T cells, total $CD3^+$ T cells and total B cells were comparable among the lean of both the strains. However, the $CD4^+$ helper T cells were significantly higher in WNIN/Gr-Ob lean compared to WNIN/Ob lean. The splenic proliferative response of unstimulated cells and proliferative response to mitogen in lean of WNIN/Gr-Ob was significantly higher compared to the lean of WNIN/Ob.

CONCLUSION

There were altered T cell subsets and B cells in both the strains and sexes. However the splenic proliferative response to mitogen was decreased only in WNIN/Gr-Ob male animals.

3 IMMUNE STATUS OF WNIN MUTANT OBESE RATS WITH REFERENCE TO LEPTIN AND OBESITY (IMMUNE RESPONSE TO HEPATITIS B VACCINE)

Clinical and epidemiological data support the evidence that the incidence and severity of infectious illnesses is higher and wound healing is poor in obese. However, few studies have also reported decreased antibody response to vaccines. It was shown that in adults and in children the antibody responsiveness to hepatitis B vaccine in obese individuals was reduced. And in another study there was reduced antibody response to tetanus vaccine in overweight children. However, there are no studies on the immune response to vaccines in obese animal models. This is the first study to observe the immune response to vaccination in obese animal model.

To fulfill this objective three months old obese and lean counterparts of WNIN/Gr-Ob and WNIN/Ob female animals were vaccinated with Hepatitis B vaccine (4µg-intramuscular injection). After one week of booster dose administration the blood was collected for estimating serum IgG levels and animals were sacrificed to collect spleen and peritoneal macrophages. As a measure of humoral immunity the serum IgG levels were determined whereas splenic lymphocyte proliferative response to mitogen as well as to specific mitogen was studied as a measure of cell mediated immune response. Peritoneal macrophage activity was also studied which was indicative of innate immunity.

RESULTS

WNIN/Gr-Ob:

Splenic lymphocyte proliferation to mitogen (Fig 8A): The proliferative response of unstimulated lymphocytes in obese control and obese vaccinated ranged from 1815-8490, 3955-10540 CPM and in lean control and lean vaccine it ranged from 3055-7890, 3970-11420 CPM. Whereas the proliferative response to mitogen in obese control and obese vaccine ranged from 55340-171865, 64900-476640 CPM and in lean control and lean vaccine it ranged from 72925-278825, 143570-609310 CPM. In the control and vaccine treated obese and lean animals the splenic lymphocyte proliferative response of unstimulated cells was comparable. The splenic lymphocyte proliferative response to concavalin A was comparable between obese and lean controls whereas administration of vaccine increased the splenic lymphocyte proliferative response in lean animals but not in obese vaccinated.

Splenic lymphocyte proliferation to Hepatitis B surface antigen (HBsAg) (Fig 8B): The proliferative response to HBsAg in obese control and obese vaccine ranged from 2685-9015, 2730-9040 CPM and in lean control and lean vaccine it ranged from 1965-8670, 2765-13010 CPM. Administration of vaccine increased the splenic lymphocyte proliferative response to HBsAg in lean animals but not in obese vaccinated.

Antigen specific antibody response (Fig 9): The O.D values for IgG ranged from 0.86-1.41 in obese vaccine whereas in lean vaccine it ranged from 1.054-2.2 O.D. The HBsAg specific IgG response was observed in both obese and lean animals one week after the booster dose. However the antibody titre was significantly higher in lean compared to obese.
Peritoneal macrophage nitrate production (Fig 10): The amount of nitrate released from unstimulated macrophages in obese control and obese vaccine ranged from 1.8-2.9 and 0.39-4.2 ng/ml and in lean control and lean

Fig: 8A

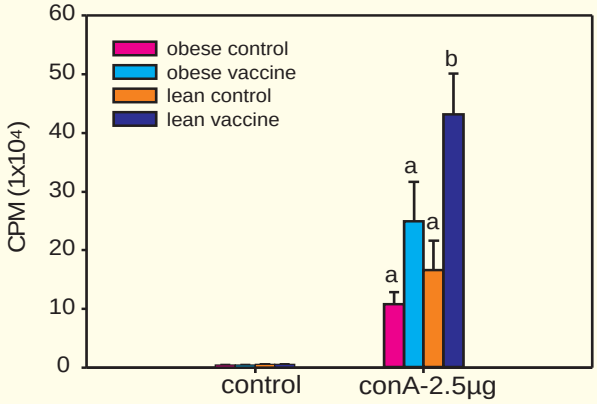


Fig: 8B

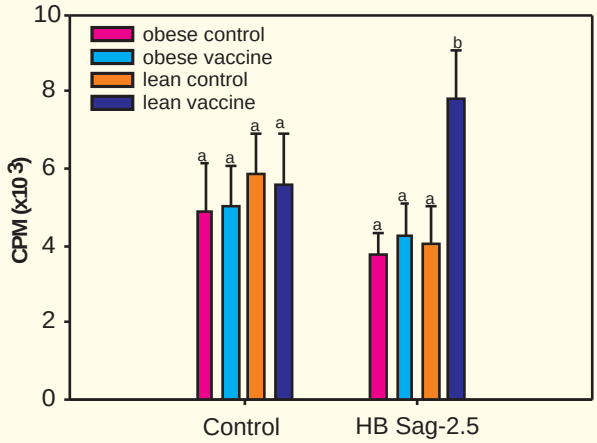


Fig 1: Proliferation of splenic lymphocytes was studied in obese and lean of 3 months old WNIN/Gr-Ob females by incorporation of ^{3}H thymidine and is expressed as counts per minute. 1A: with concavalin A at 2.5µg/2x10^{6} cells; 1B: with HBsAg at 2.5µg/2x10^{6} cells. Values are mean ± S.E; obese and lean controls n=6; obese and lean vaccine n=8. Differences between groups were analyzed by a one way analysis of variance at $P<0.05$. Means that don't share a common letter are significantly different.

vaccine it ranged from 0.26-1.39 and 1.3-5.4ng/ml. whereas the amount of nitrate released upon stimulation with LPS ranged in obese control and obese vaccinated ranged from 3.05-5.03 and 0.93-8.95 and in lean control and lean vaccine it ranged from 0.97-2.6 and 2.9-6.87 ng/ml. Nitrate production by unstimulated macrophages and macrophages upon stimulation with LPS was significantly

higher in obese control compared to lean control. However, vaccine treatment increased the nitrate production in lean but not in obese.

Fig: 9A

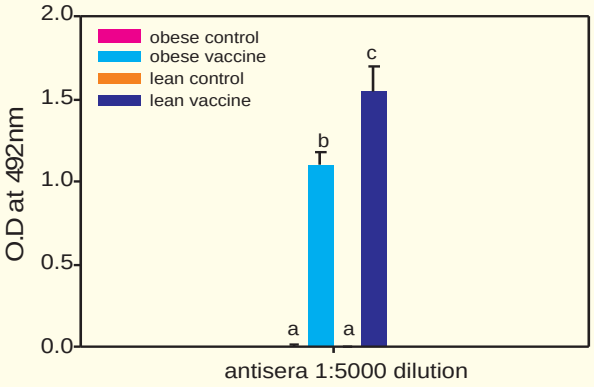


Fig 9: HBsAg specific IgG response was estimated by ELISA in 1:5000 antisera dilution after 1 week of second dose Hepatitis B vaccine administration in obese and lean of 3 months old WNIN/Ob females. Values are mean ± S.E; obese and lean controls n=6; obese and lean vaccine n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share a common letter are significantly different.

Fig: 10

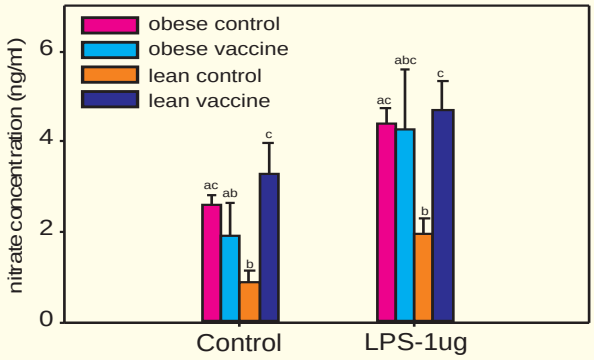


Fig 10: The amount of nitrate released was estimated in the culture supernatants of unstimulated peritoneal macrophages and in macrophages stimulated with LPS at a concen-tration of 1µg/1x10^6 cells in obese and lean WNIN/Gr-Ob. Values are mean ± S.E; obese and lean controls n=6; obese and lean vaccine n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share a common letter are significantly different.

WNIN/Ob:

Splenic lymphocyte proliferation to mitogen (Fig 11A): The proliferative response of unstimulated lymphocytes in obese control and obese vaccinated ranged from 3585-14445, 3035-6510 CPM and in lean control and lean vaccine it ranged from 3485-10770, 2425-9445 CPM. Whereas the proliferative response to mitogen in obese control and obese vaccine ranged from 9820-774160, 40415-733020 CPM and in lean control and lean vaccine it ranged from 231380-690735, 171940-813495 CPM. In the control and vaccine treated obese and lean animals the splenic lymphocyte proliferative response of unstimulated cells and proliferative response to mitogen was comparable.

Splenic lymphocyte proliferation to Hepatitis B surface antigen (HBsAg) (Fig 11B): The proliferative response to HBsAg in obese control and obese vaccine ranged from 1680-5720, 3355-12350 CPM and in lean control and lean vaccine it ranged from 1220-6745, 1855-34175 CPM. Administration of vaccine increased the splenic lymphocyte proliferative response to HBsAg in lean animals but not in obese vaccinated.

Antigen specific antibody response (Fig 12): The O.D values for IgG ranged from 1.55-3.07 in obese vaccine whereas in lean vaccine it ranged from 1.13-3.53 O.D. HBsAg specific IgG response was observed in both obese and lean animals one week after the booster dose. However the antibody titre was significantly higher in lean compared to obese.

Peritoneal macrophage nitrate production (Fig 13): The amount of nitrate released from unstimulated macrophages in obese control and obese vaccine ranged from 3.0-5.0 and 1.01-5.5 ng/ml and in lean control and lean vaccine it ranged from 2.6-6.2 and 2.5-8.1 ng/ml. whereas the amount of nitrate released upon stimulation with LPS ranged in obese control and obese vaccinated ranged from 4.4-6.5 and 3.77-5.4 and in lean control and

lean vaccine it ranged from 3.6-7.2 and 1.9-8.7 ng/ml. Peritoneal macrophage nitrate production was comparable between obese and lean and it didn't increase with Hepatitis B vaccine.

Fig: 11A

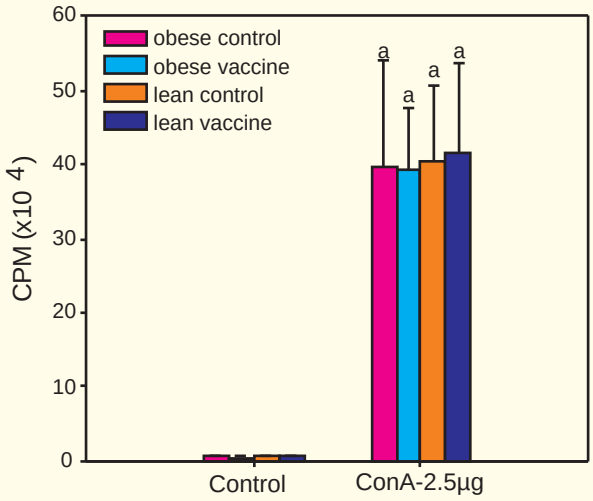


Fig: 11B

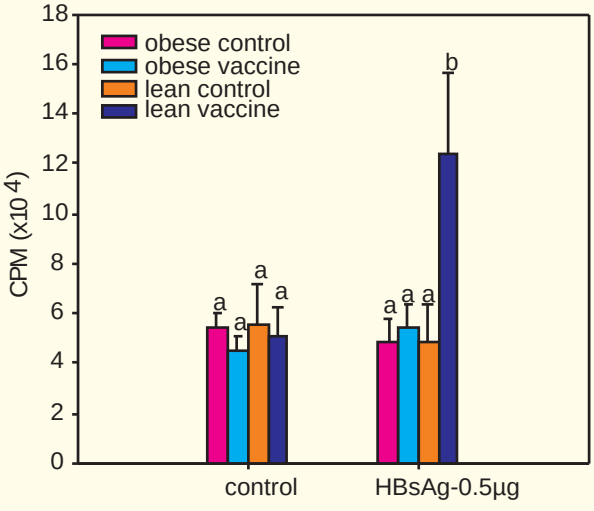


Fig 11: Proliferation of splenic lymphocytes was studied in obese and lean of 3 months old WNIN/Ob females by incorporation of ^{3}H thymidine and is expressed as counts per minute. 11A: with concavalin A at 2.5µg/2x10^{6} cells; 11B: with HBsAg at 2.5µg/2x10^{6} cells. Values are mean ± S.E; obese and lean controls n=6; obese and lean vaccine n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share a common letter are significantly different.

Fig: 12

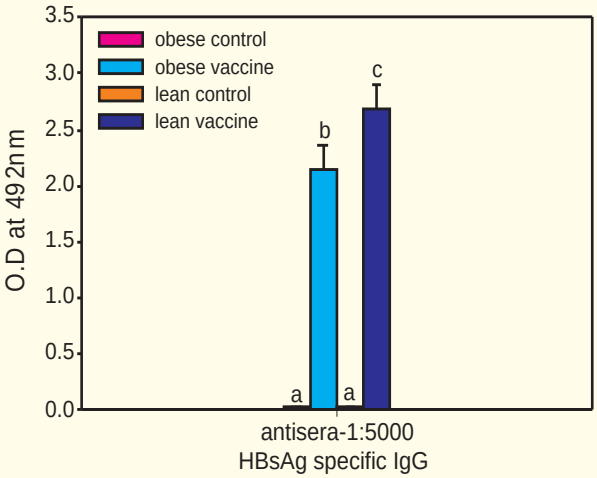


Fig 12: HBsAg specific IgG response was estimated by ELISA in 1:5000 antisera dilution after 1 week of second dose Hepatitis B vaccine administration in obese and lean of 3 months old WNIN/ Ob female animals. Values are mean ± S.E; obese and lean controls n=6; obese and lean vaccine n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share a common letter are significantly different.

Fig: 13

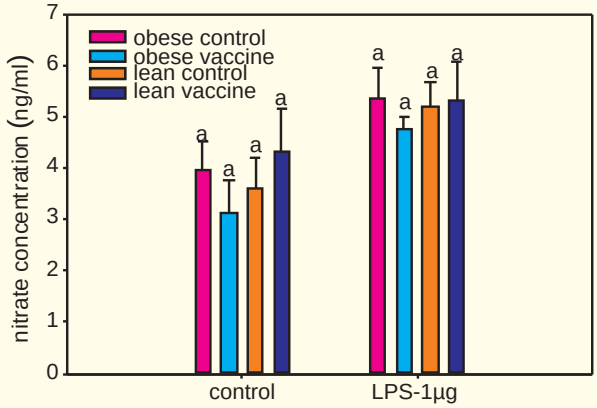


Fig 13: The amount of nitrate released was estimated in the culture supernatants of unstimulated peritoneal macrophages and in macrophages stimulated with LPS at a concentration of 1µg/1x10^{6} cells in obese and lean WNIN/Ob. Values are mean ± S.E; obese and lean controls n=6; obese and lean vaccine n=8. Differences between groups were analyzed by a one way analysis of variance at P<0.05. Means that don't share a common letter are significantly different.

Across strains

The splenic proliferative response to mitogen at 2.5µg/2x10^6 cells was significantly higher in WNIN/Ob lean control compared to WNIN/Gr-Ob lean control, whereas among other groups they were comparable. Similarly, the splenic proliferative response to HBsAg was higher in WNIN/Ob lean vaccine animals compared to WNIN/Gr-Ob lean vaccine, whereas it was comparable among other groups. The HBsAg specific IgG response and Nitrate production by unstimulated and stimulated (by LPS) macrophages were higher in WNIN/Ob obese and lean compared to WNIN/Gr-Ob obese and lean animals.

CONCLUSIONS

1. The HBsAg specific IgG levels were significantly low in both WNIN/Gr-Ob (30% reduction) and WNIN/Ob (20% reduction) obese compared to lean.
2. The splenic proliferative response to mitogen and HBsAg in WNIN/Gr-Ob was low in obese when compared to lean. In WNIN/Ob the splenic proliferative response to HBsAg only was lower in obese compared to lean.
3. The peritoneal macrophage function as assessed by the amount of nitrate released was altered in WNIN/Gr-Ob, whereas it was unaltered in WNIN/Ob animals.

III. BASIC STUDIES

1 ISOLATION AND IDENTIFICATION OF HUMAN MILK FACTOR THAT ENHANCES IRON BIOAVAILABILITY: AN EXPLORATORY STUDY

It is known that the bioavailability of iron from human milk (HM) is greater than that of cow's milk (CM) in infants and fasted adults. However, factors responsible for the greater Fe bioavailability of HM have not been identified. Recent evidences suggest that a low molecular weight human milk whey factor increases the bioavailability of ferric iron. Although poorly characterized, this factor appears to bind and solubilize ferric iron and remains stable after simulated *in vitro* gastric and intestinal digestion. Therefore, the objective was to identify and characterize this factor which may have immense importance in increasing the bioavailability of iron in food fortification formulations.

METHODOLOGY

Sample processing and ultrafiltration: Approval of the Institutional Ethical Committee of Gandhi Hospital, Hyderabad was obtained to collect about 10-15mL of breast milk from each mother. Typically, 40-50 mL of pooled milk sample was centrifuged at 4000g for 30 min, and the upper fat layer was removed to obtain milk whey (HM whey). The HM whey was further delipidated with equal volumes of ether and subjected to ultrafiltration using 10kDa cutoff membrane to generate filtrate (10kF) and retentate (10kR).

Gel filtration chromatography: The 10kF fraction was purified on Superdex peptide 10/30 HR column and the fractions were collected and immediately used for the iron solubilization and ferric reductase assays.

Iron solubility assay*:* Milk fractions were diluted in buffer and incubated with 25 µM iron ($FeCl_3$) traced with ^{59}Fe at 37°C for 1 h. The radioactivity in the supernatant was counted using a gamma counter. A blank (MES buffer), Fe with ascorbic acid (1:20 ratio) and 1M HCl (100% solubility) were also run simultaneously.

Ferric reductase assay: Ferric reductase assay was performed by monitoring the binding of ferrous iron to the ferrozine, using colorimetric (562 nm) method. Briefly, the reaction was carried out in a micro well plate with milk fractions and 50 µmole/L ferric iron from different chemical sources (ferric chloride, ferric nitrate, ferric citrate, ferric pyrophosphate and ferric EDTA) and ferrozine at 25°C for 10 min. The formation of ferrous iron was calculated from a standard curve of ammonium ferrous sulfate and the specific activity of ferric reductase expressed as moles of ferrous iron formed/min/mg protein.

Caco-2 cell uptake studies: In order to further test whether the milk fractions also increased the absorption of ferric iron, differentiated Caco-2 cells were incubated with ferric iron (25 µM traced with carrier free ^{59}Fe) in the presence and absence of various milk fractions and ascorbic acid (1:20 ratio).

Statistics*:* The results were subjected to One-way ANOVA followed by least significant differences (LSD) test, using SPSS package (Version 11.0). The results were considered significant if the P value was <0.05.

RESULTS

The solubility of ferric iron was negligible (5%) in the absence of acid (1M HCl), ascorbic acid or milk fractions. All the milk fractions significantly enhanced the iron solubility with 10kF fraction exhibiting solubility comparable to ascorbic acid (Fig.14 A). Of the three fractions tested for the ferric reductase activity

only in 10kF fraction showed (7.5 times) higher activity compared to the other two fractions (Fig. 14B).

In line with these results, the 10kF showed maximum iron uptake in Caco-2 cells (Fig. 14C).

To further characterize the 10kF fractions, gel filtration chromatography was performed on a superdex-peptide column. Gel filtration profile of 10kF fraction showed 5 distinct peaks at 280 nm (Fig. 15 A, solid line) with the first peak eluting at apparent molecular mass of 1500 Da (eluting just after the cyanocobalamine, MW 1579 Da, Fig. 15A, dotted line). Further characterization of this peak showed both ferric reductase (Fig. 15B) and iron solubilization activities (Fig. 15C).

Fig. 14A

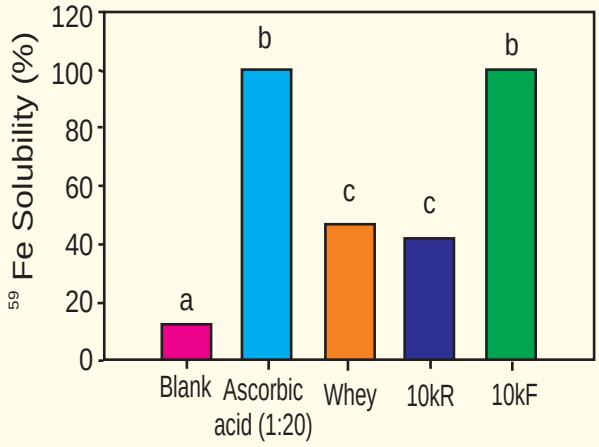


Fig. 14B

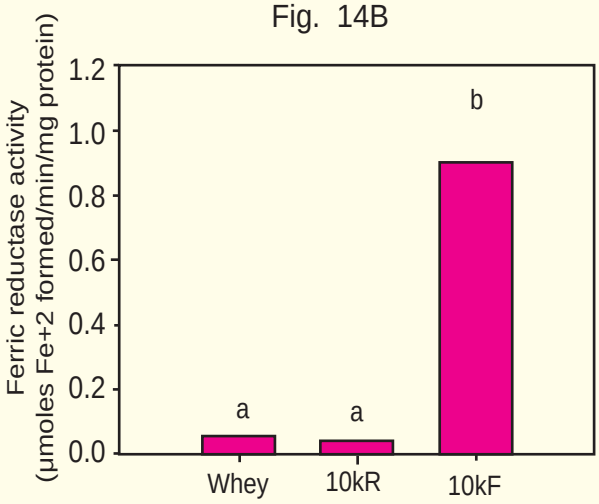


Fig. 14C

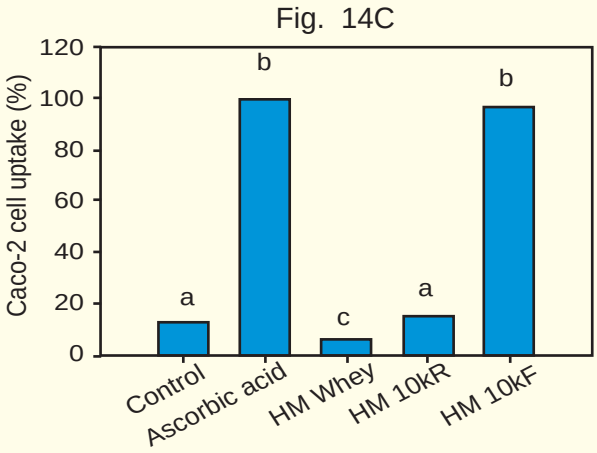
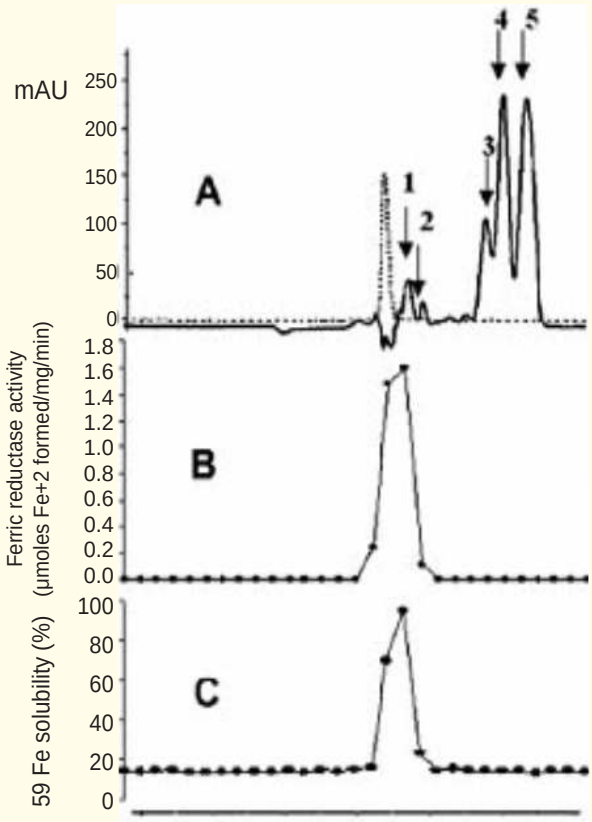


Fig 14: Effect of various milk fractions on ferric iron solubility (A) and Ferric reductase activity (B).The percentage solubility was calculated by considering the solubility of $FeCl_3$ added directly to the 1M HCl as 100%. Effect of various milk fractions on Caco-2 cell iron uptake (B). The percentage uptake was calculated by considering the uptake in the presence of ascorbic acid as 100%. The data was analyzed by One-way ANOVA and the results considered significant if $P<0.05$.



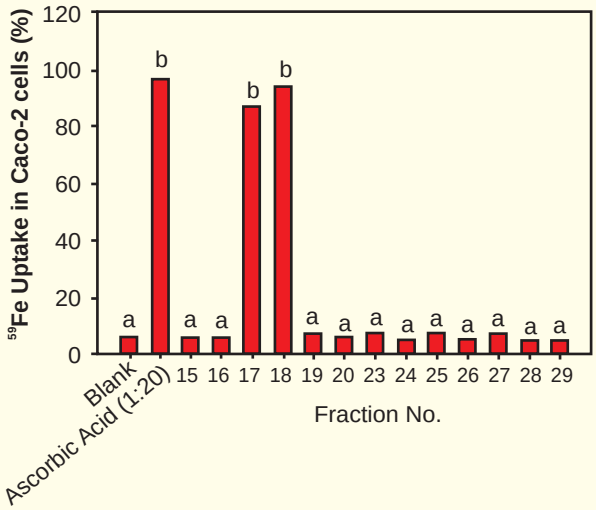


Fig 15: Superdex peptide gel filtration chromatography of 10kF human milk fraction: 0.5 mL of the 10kF fraction (panel A, solid line) or cyanocobalmine (Panel A, dotted line) was fractionated on Superdex peptide column and eluted at a flow rate of 0.5 mL/min while monitoring the absorbance at 280nm. The arrows 1-5 indicate the peak nos. All the fractions were assayed for ferric reductase activity (panel B) and iron solubilization activities (panel C) and uptake of iron in Caco-2 cells (panel D). The bars indicate mean +SD & bars with different letters differ significantly at $P<0.05$.

The other peaks (denoted 2–5) though had appreciable absorbance at 280 nm, showed negligible ferric reductase and iron solubilization activities. Among the various fractions tested for iron uptake in Caco-2 cells only the 17th and 18th fractions, possessing ferric reductase activity, enhanced the uptake of iron in Caco-2 cells (Fig.15D).

CONCLUSIONS

In conclusion, for the first time ferric reductase activity in low molecular weight human milk fractions and provided evidences for enhanced ferric iron solubility and uptake in Caco-2 cells were demonstrated. The UV light absorption at 280 nm coupled with enzymatic activity strongly suggest that the milk factor is indeed a protein or a peptide.

2 BIOLOGICAL SIGNIFICANCE OF PHYTOFERRITIN: GASTRIC STABILITY OF PEA FERRITIN AND GOAT LIVER FERRITIN *IN VIVO* IN RATS AND *IN VITRO* WITH HUMAN GASTRIC JUICE

Ferritins are iron storage proteins ubiquitously present in all organisms. Given the capability of ferritin to store 4500 iron atoms in its mineral core, has been considered as a target molecule for biofortification to increase iron density in staple foods. However, the bioavailability of ferritin iron is controversial. Recently it was demonstrated at NIN that purified pea seed ferritin is sensitive to simulated gastric pH conditions and its iron bioavailability is modulated by dietary factors such as ascorbic acid and phytic acid in Caco-2 cell model. These results suggested that the enterocyte absorption of ferritin iron is similar to non-heme iron.

Thus the objective of the study was to assess the digestive stability of pea seed and goat liver ferritins under *in vivo* conditions in rats and *in vitro* with human gastric juices.

METHODS

In vivo studies in rat: Approval for the use of 24 Wistar NIN male rats of body weight 250g was obtained from the Institutional Animal Ethics Committee of NIN. Twenty four hours fasted rats were orally administered pea ferritin or goat liver ferritin (1mg/mL) by gavage. Rats were killed at 0, 15, 30, 60 min (n=3) for each time point and the contents of stomach were collected from the cardiac sphincter end directly into tubes containing PAGE sample buffer along with protease inhibitor cock-tail. The contents were centrifuged and the supernatants subjected to 6 % PAGE and the gel stained for iron and protein.

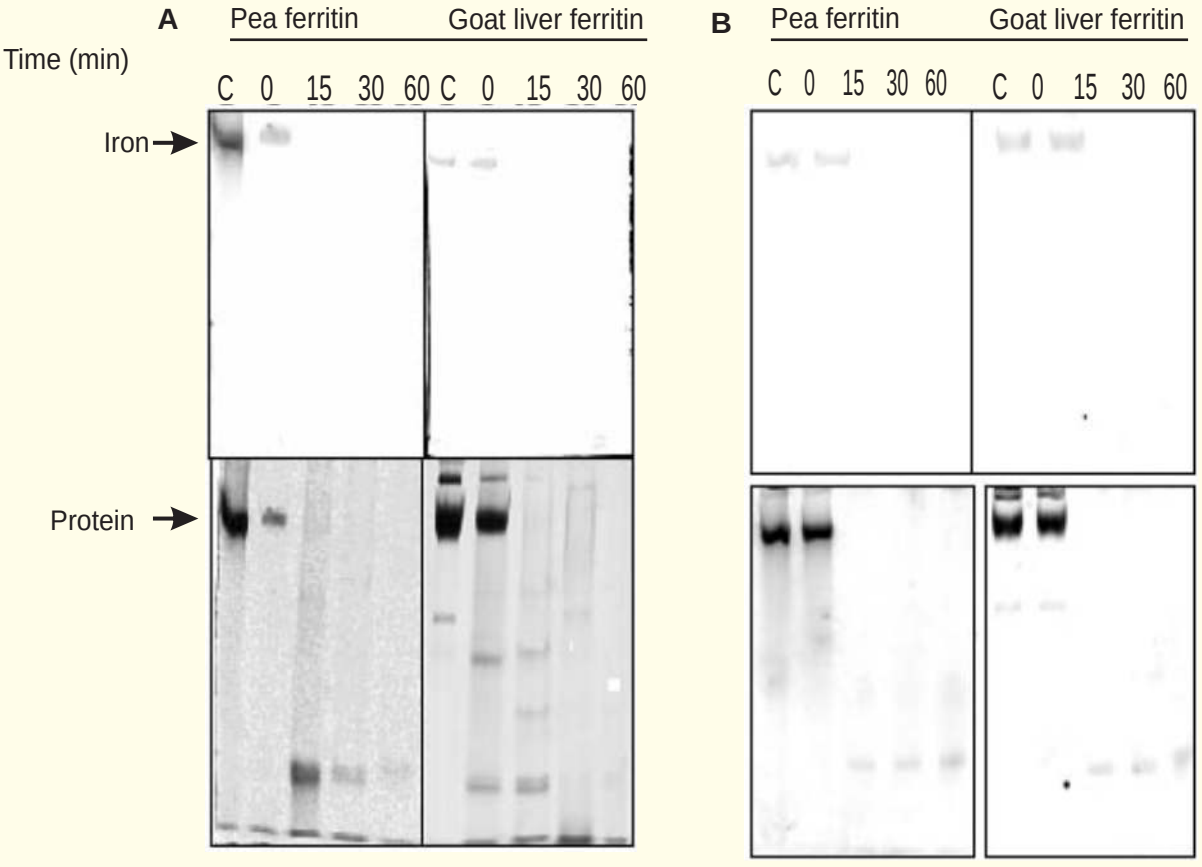
In vitro studies with human gastric juice: Gastric contents were collected from the stomach from (n= 6) healthy subjects, mean age 40 years, attending the Asian Institute of Gastroenterology, Hyderabad undergoing endoscopy under fasting conditions. Measurements of pH and pepsin activity were assessed. About 100 µg of goat liver and pea ferritin was incubated with gastric juice at 37^{0}C for various time points and analyzed for protein or iron.

RESULTS

In vivo stability in rats: Iron stain associated with ferritin protein was not observed at any of the time points tested both with pea or goat ferritin (**Fig 16 Panel A**). A low molecular weight protein band was seen during the same time course which decreased in a time dependent manner.

In vitro stability to human gastric juice: The pH of the human gastric juice varied between 2.1 to 2.4 and the pepsin activity between 20-80 units/mg protein among 6 subjects. Incubation of either pea or goat liver ferritin with human gastric juices revealed disappearance of iron and protein stain in a time dependent manner (Fig 16 Panel B).

Fig 16: Native PAGE (6%) of digest stained for iron (top panel) or protein (bottom panel).
[Digestive stability of ferritin in vivo in rats (A) in vitro with human gastric juice (B)]



CONCLUSIONS

Pea seed ferritin and goat liver ferritin release iron at gastric pH and gastric enzymes further fastens this process, suggesting that iron bioavailability from ferritin is not different from that of non-heam iron.

3 STUDIES ON MECHANISM OF ABSORPTION AND CYTOPROTECTIVE EFFECTS OF ZINC IN CACO-2 CELL (HUMAN COLON CARCINOMA CELLS) INTESTINAL MODEL

Deficiencies of iron and zinc co-exist in developing countries, including India. Strategies to control their deficiencies include therapeutic supplementation or food fortification of either singly or in combination. It is known that these minerals interact and therefore the issue of combined supplemen-tation has been controversial. Studies from NIN a rat model have shown that iron induced oxidative stress can be abolished by co-supplementation with zinc. However, the down stream events of the zinc modulation of iron induced oxidative stress has not been elucidated. Oxidant induced activation of aconitase/iron regulatory protein (IRP) pathway such as the induction of iron regulatory proteins 1 and 2 their activities are known. Thus, It was hypothesized that zinc inhibits oxidant induced iron uptake by modulating the aconitase-IRP system.

The objective was to test the effects of zinc on glucose-oxidase induced oxidative stress and the associated mechanisms in Caco-2 cell line (enterocyte) model.

METHODS

Induction of oxidative stress: All experiments were performed in Caco-2 cells grown in six well plates, after 14 days. Oxidative stress was induced in Caco-2 cells by treating with 60 mU of glucose oxidase (GO) for generating H_2O_2.

To monitor the DCF fluorescence as a measure of intracellular oxidative stress, ***Caco-2*** cells were treated with GO either in the presence or absence of zinc for 0-12 h followed by incubating with 10 µM DCF-DA for 15 min. The formation of intracellular DCF was monitored using a fluorescence microscope equipped with FITC filter (Nikon-TE 2000S). The anti-oxidant status of the cell was determined using GSH: GSSG ratio in cell lysates.

Iron uptake was assessed by incubation of cells with ^{59}Fe (ferric chloride; 0.1 µCi) in the presence of 60 mU of GO for 0 to 8 h and counting the cell associated radioactivity.

DNA ladder assay: Caco-2 cells were treated with 60 mU /mL of GO in the presence or absence of zinc (25 and 50 µM) for 24 h. The cell lysate were directly loaded on the 2% agarose gel containing 1 µg/ml ethidium bromide and visualized using Gel-doc system (Bio Rad).

Caspase-3 activity, Bcl-2, Bax, DMT- 1, IRP-1 and metallothionein (MT): For the analysis of caspase-3 activity, Bax and Bcl 2 expression, the cells were treated as above for 18 h and for DMT 1, IRP 1 and metallothionein; cells were incubated for 8 h. The cells were re-suspended in RIPA buffer containing protease inhibitor cocktail, lysed and collected the supernatant and trans-blotted (Bax, Bcl-2, MT, DMT-1 and GAPDH). In the case of IRP-1 and IRP-2, cells were lysed and subjected to differential centrifugation at 1,00,000 g to isolate polysomes. Pellet was re-suspended in buffer containing triton X-100 and about 100µg of the protein used for immunoblotting. Immunoblotting was done with respective primary antibodies (rabbit anti-human DMT-1, IRP-1, Bcl-2, Bax, GAPDH and hrMT polyclonal). The protein bands were detected using the ECL method (Amersham Biosciences).

Aconitase activity was determined in 12,000g supernatant by following the disappearance of cis-aconitate at 240 nm expressed as units/mg protein. One unit is defined as µ mole of cis-aconitate disappeared/min.

Effect of extra cellular iron chelator BPDS on GO induced oxidative stress and apoptosis: H_2O_2 induced iron signaling and apoptosis was studied either in the presence or absence of 50 µM of zinc or 5 mM of batho-

phenanthroline disulfonicacid (BPDS) an extra cellular iron chelator.

Intra cellular labile iron and ferritin expression: For measuring intra cellular labile iron pools, cells were incubated as above for 8 h followed by incubating with 10 µmole/L iron specific fluorescent probe Phen Gren-SK. Cells were lysed in 2 % triton X-100 and the fluorescence intensity was measured in Cary-Eclipse spectrofluorimeter. Ferritin expression was assessed by sandwich ELISA method.

RESULTS

Zinc along with GO reduced the DCF fluorescence (Fig.17A) while it increased the GSH: GSSG ratio (Fig. 17B). Zn dose dependently reduced GO induced DNA fragmentation (Fig.17C), suggesting inhibition of apoptosis.

In order to understand the mechanism by which Zn inhibits the apoptosis, caspase-3 activity, Bcl-2 and Bax levels were measured. Co-incubation of Zn reversed GO induced increase in the activity of caspase-3 (Fig. 17D) and reduced the expression of Bcl-2 without altering the expression of pro-apoptotic protein Bax (Fig 17E).

Zinc inhibits GO induced iron signaling: GO increased the uptake of iron which was inhibited upon co-treatment with zinc (Fig. 18A). There was an increase in DMT-1 in the presence of Zn (Fig. 18B). The presence of GO reduced aconitase activity (Fig. 18E) while increasing the RNA binding activity of IRP-1and IRP-2 (Fig.18D). GO+Zn further reduced the activity of aconitase and inhibited RNA binding activity of IRP-1 and IRP-2. Under these conditions Zn was able to increase the cellular ferritin levels (Fig. 18C), suggesting sequestration of labile iron in the presence of Zn.

Effect of extra cellular iron chelator BPDS on GO induced apoptosis and iron signaling: Treatment of Caco-2 cells with 5 mM BPDS reduced the GO-induced iron

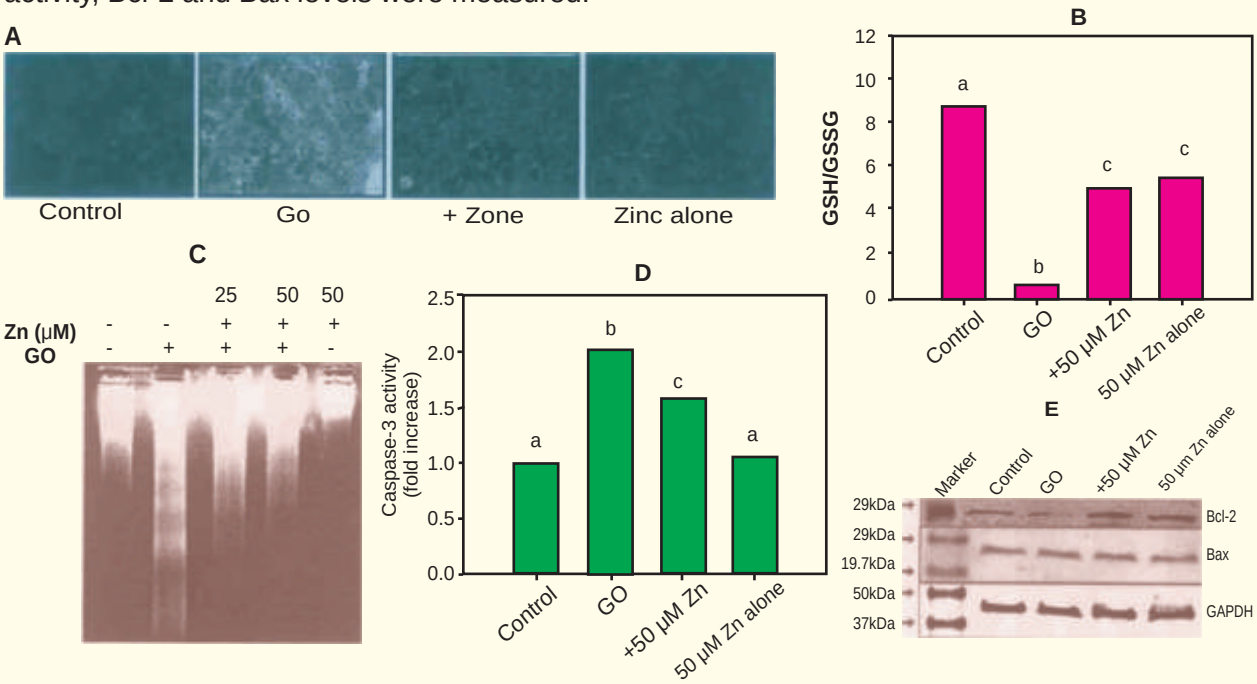


Fig 17. Effect of zinc on GO induced changes in cellular oxidant and antioxidant balance. DCF fluorescence (A), GSH: GSSG ratio (B). DNA ladder (C), caspase-3 activity (D) and immunoblots of anti and pro-apoptotic Bcl family proteins Bax and Bcl-2 (E). Bars represent mean+SD of three independent observations. The bars that do not share common superscript differ significantly at $P<0.05$.

uptake (Fig. 19A), DCF fluorescence (Fig. 19B), apoptosis (both DNA fragmentation and *Bcl-2* levels) (Fig.19C&D) & IRP-1 activation (Fig. 19D) similar to zinc. Further BPDS up-regulated the expression of metallothionein similar to that with Zn treatment (Fig. 19D). However, unlike Zn, BPDS treatment reduced cell ferritin content (Fig. 19E). Further treatment of Caco-2 cells with GO increased intracellular iron which normalized both with zinc or BPDS (Fig. 19F).

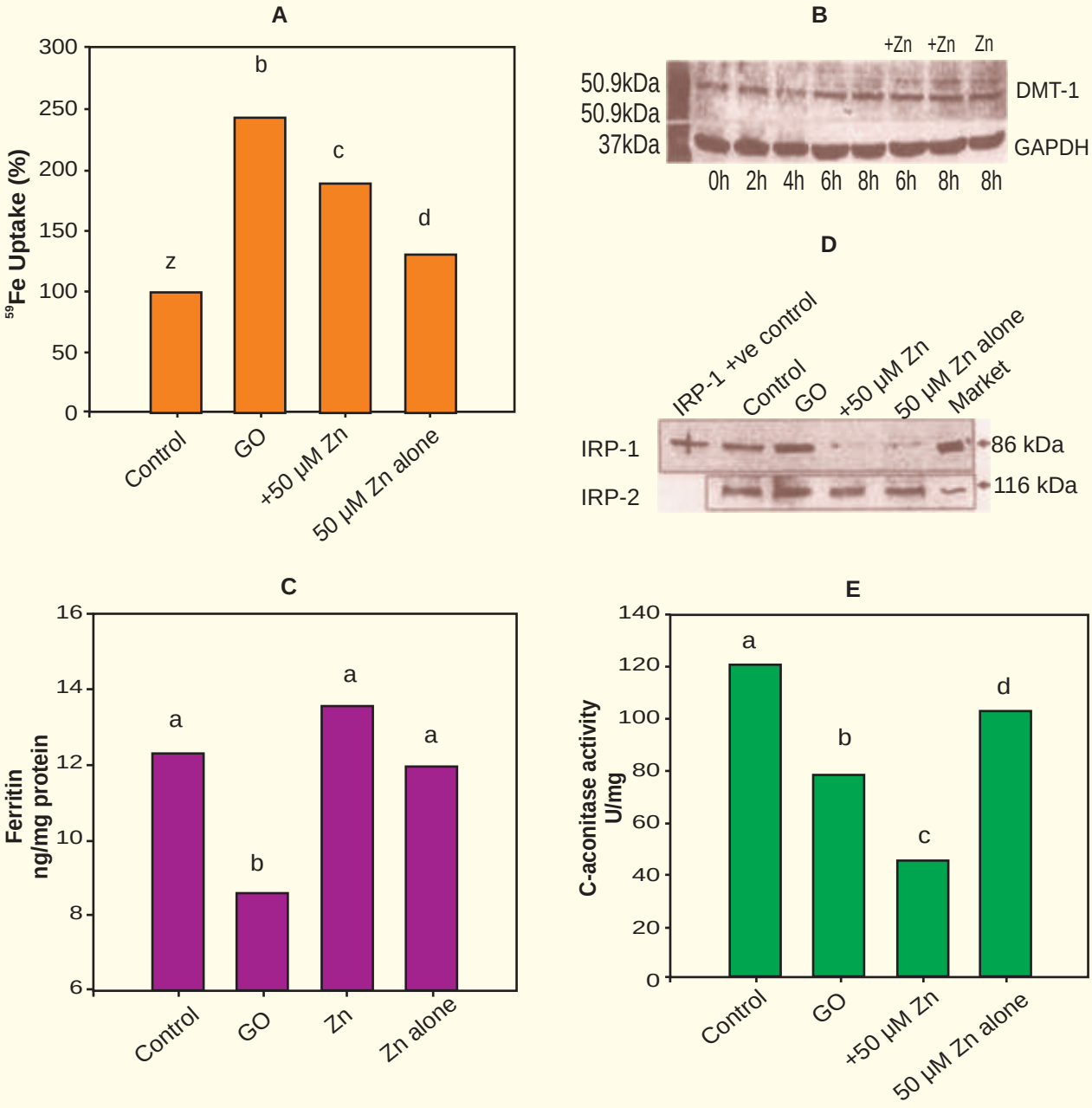


Fig 18. Effect of zinc on GO induced cellular changes. ^{59}Fe uptake (A), immunoblots of DMT-1 (B) and RNA bound IRP-1 and IRP-2 (D), c-aconitase activity (E), ferritin levels (C). The bars that do not share common superscript differ significantly at $P<0.05$.

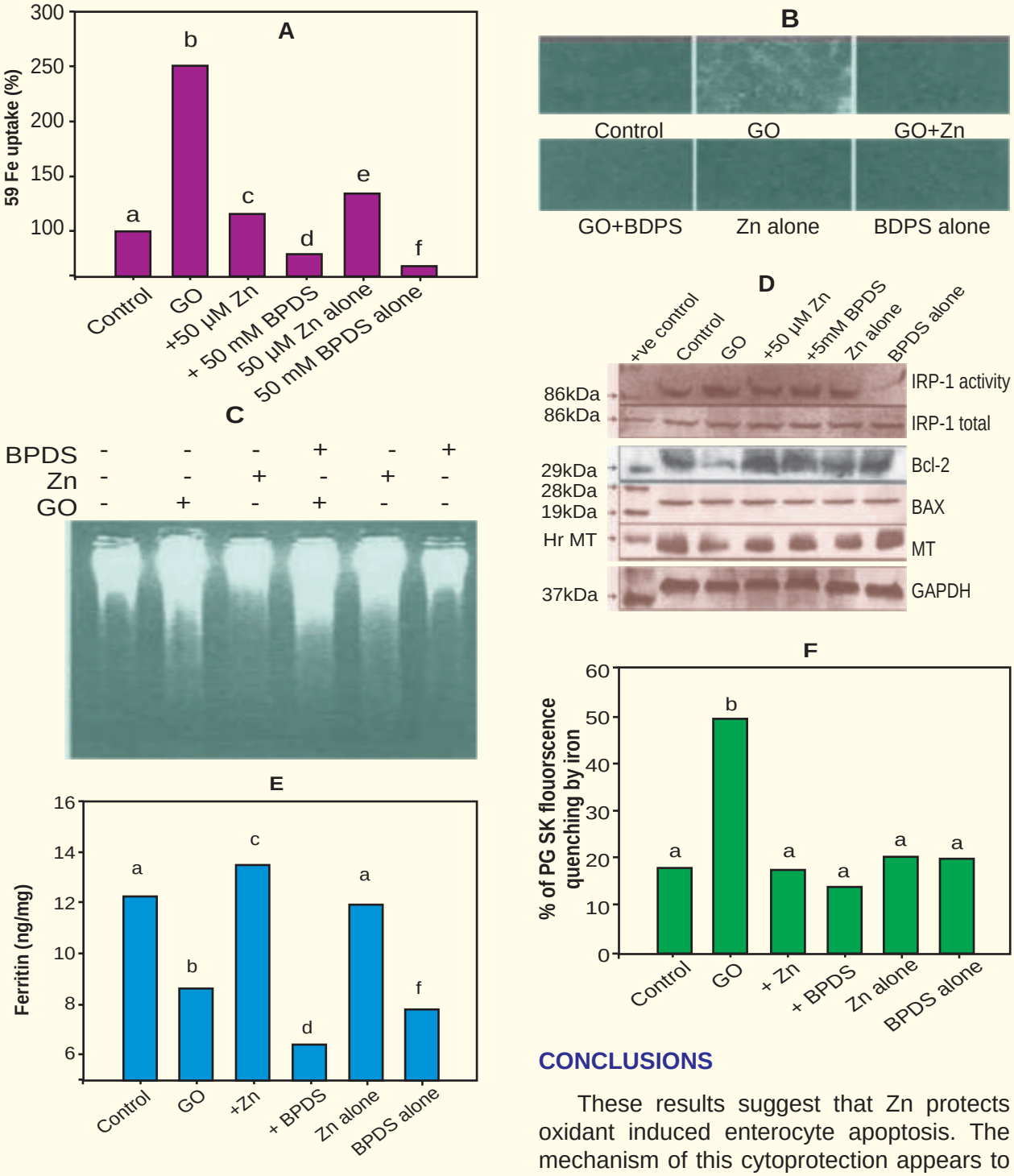


Fig 19. Effect of BPDS on GO induced oxidative stress. Iron uptake (A), DCF-DA oxidation (B), DNA fragmentation (C), immunoblotts of IRP-1, total IRP, Bcl-2, Bax and MT (D), ferritin levels (E), intracellular labile iron (F). The bars represent mean±SD and bars that do not share common superscript differ significantly at P<0.05.

CONCLUSIONS

These results suggest that Zn protects oxidant induced enterocyte apoptosis. The mechanism of this cytoprotection appears to be through the inhibition of iron signaling by inactivation of aconitase/IRP switch. Zinc reduces oxidant induced labile iron by decreasing its uptake which is required for modulating the aconitase/IRP switch and ferritin levels.

4 VALIDATION OF ZINC UPTAKE IN CACO-2 CELLS FOR BIOAVAILABILITY OF ZINC IN HUMANS: POLYPHENOL-RICH BEVERAGES ENHANCE ZINC UPTAKE AND METALLOTHIONEIN EXPRESSION IN CACO-2 CELLS

Previous studies from our lab have shown that tannic acid enhances the uptake of zinc in Caco-2 cells *(Sreenivasulu et al., 2008, J. Ag. Food Chem. 56, 10967-10972).* Other studies either in animals or in humans reported in the literature suggest that polyphenols, unlike that of iron, do not affect the zinc absorption. However, long term-consumption of green tea rich in polyphenols has been shown to increase serum and tibia zinc concentration in rats. Increased expression of metallothionein in colon of rats supplemented with tannic acid reported. Consumption of alcohol is known to cause zinc deficiency in humans, while the negative effect of alcohol on zinc uptake appears to be moderated by other components in alcoholic beverages. Therefore, the objective was to assess the effect of polyphenol rich beverages and representative polyphenols on Caco-2 cell zinc uptake.

METHODS

Cell culture: For uptake experiments, Caco-2 cells were grown in 6-well plates seeded at a density of 50,000 cells/cm^2 and used 12-14 days of post confluency.

Food matrix and beverages: Food matrices used for the study were cooked-rice, and polyphenol- rich beverages red wine (RW) containing 12% alcohol, red grape juice (RGJ) without additives, and green tea extract (GT) (1 pouch in 120 mL of boiling water for 10 min and filtered). Solid phase extraction (SPE) method on a SEP pack (10X10 mm) C-18 column (Extra SEP C-18; Sigma) was used for extracting polyphenol from red wine *[Briviba,et al., 2002, J. Nutr. 132, 2814-2818].*

In vitro digestion and Caco-2 cell zinc uptake: One g of lyophilized rice powder was traced with 5 Ci ^{65}Zn and subjected to the simulated *in vitro* digestion in the absence or presence of RW, RGJ and GT or RW polyphenol extract as described previously [Sreenivasulu et al., 2008]. Two mL of the above digests was fed to the cells, incubated at 37^{o}C for 2 h and cell associated radio activity counted in Auto - counter (Perkin Elmer Wizard 1480).

Effect of representative polyphenols on Caco-2 cell zinc uptake and MT expression: ^{65}Zn uptake in the presence and absence of tannic acid, quercitin, catechin, caffeic acid and gallic acid (10:1 molar ratio) was measured as described above. Proteins in the cell lysates was also subjected to immunoblotting for metallothionein using h r MT antibody. Similar experiments were also carried out either in the presence or absence of phytic acid (1:10 molar ratio, inositol hexa phosphate, IP6), a known inhibitor of zinc absorption.

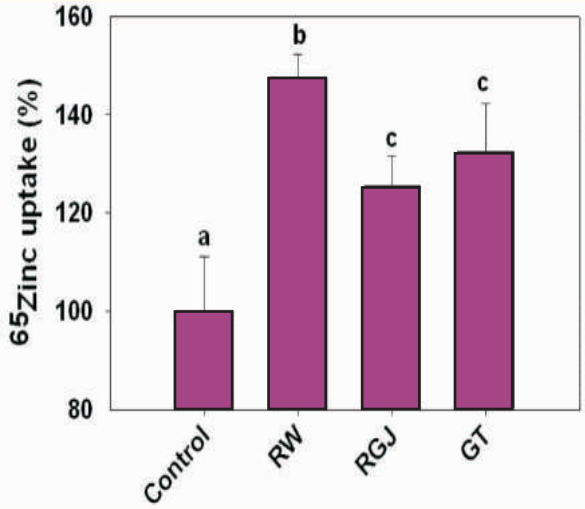
In vitro binding of zinc with polyphenols and zinquin: Zinquin binds to zinc in a mole-to-mole ratio and emits fluorescence at 490 nm. Zinc (10 µmoles/L) was pre-incubated with 0-200 µmoles/L of tannic acid, quercetin, catechin, gallic acid and caffeic acid for 30 min at room temperature followed by the addition of 10 µmole/L zinquin and fluorescence quantitated at 490 nm.

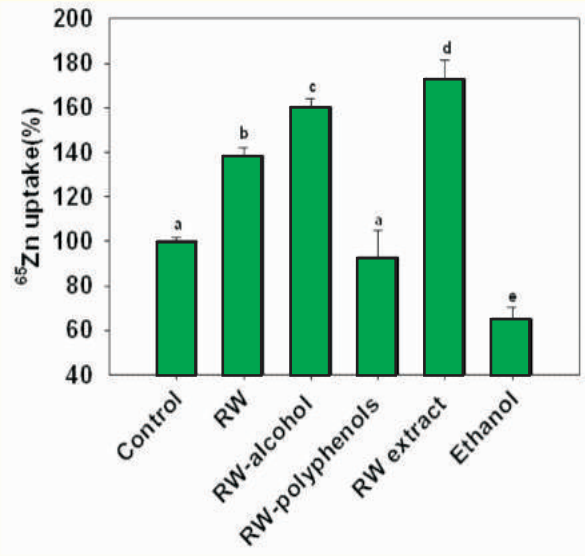
RESULTS

Effect of polyphenol rich beverages on Caco-2 cell zinc uptake: RW, RGJ, GT significantly increased the uptake of zinc from a rice-based meal compared to control (Fig. 1A). Zinc uptake was significantly higher in the presence of RW (47.36 ± 4.9%) compared to RGJ (32.4 ± 9.8 %) and GT (25.42 ± 6.13 %), which showed similar increase in uptake.

Effect of components of red wine polyphenols

and structural polyphenols on Caco-2 cell zinc uptake and MT expression: Various fractions of RW obtained from SPE showed varied effect on Caco-2 cell zinc uptake. Addition of either RW or RW without alcohol (RW evaporated under nitrogen) and RW extract significantly improved zinc uptake from rice-matrix (Fig. 1B). However, RW devoid of polyphenols and alcohol failed to enhance the uptake of zinc. Interestingly, alcohol significantly inhibited the uptake of zinc compared to control. The uptake of zinc significantly increased in the presence of tannic acid and quercetin (80.5 4.6, 133.23 34.6%, respectively above the control) while catechin, gallic acid and caffeic acid did not influence zinc uptake (Fig 1C).





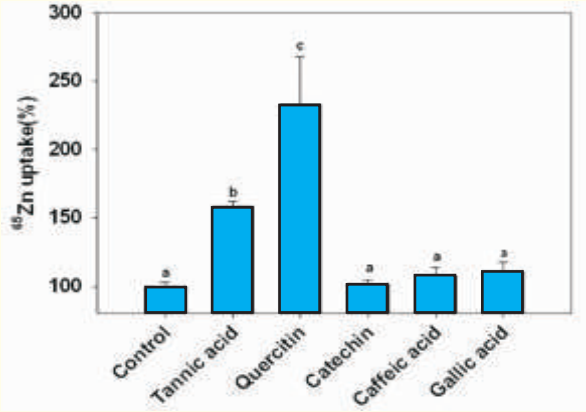


Fig 1. Effect of polyphenol-rich beverages (A), C-18 fractions of red wine (B), representative polyphenols on zinc uptake (C) on zinc uptake in Caco-2 cells from cooked rice. The percentage uptake was calculated assuming zinc uptake from rice without beverage and without representative polyphenols as 100%. Bars represent mean + SD and the bars without common superscript differ significantly (P<0.05, n=6). RW-Red wine, RGJ- Red grape juice, GT-Green tea. RW-red wine, RW-alcohol -red wine devoid of alcohol, RW-polyphenols - red wine devoid of polyphenols, RW extract – polyphenol extract from red wine, ethanol -12% ethanol

Zinc in the presence of polyphenols augmented the expression of MT (Fig. 2A). The highest expression was observed with quercitin followed by tannic acid and RW extract. Further, tannic acid alone or in the presence of zinc induced MT expression in a dose-dependent manner (Fig. 2B). Nonetheless, presence of phytic acid either alone or with tannic acid decreased the metallothionein expression

In vitro binding of zinc with polyphenols and zinquin: Zinc enhanced the zinquin specific fluorescence in a concentration dependent manner and saturated at 1:1 molar ratio (Fig 3A inset). Of the polyphenols tested, tannic acid, quercitin quenched zinc-induced zinquin fluorescence in a dose-dependent manner. There was no quenching of zinc-zinquin fluorescence with catechin, gallic acid and caffeic acid. The quantum yield in fluorescence spectra was also found to be

minimum with quercitin and was 50% with tannic acid (Fig. 3B).

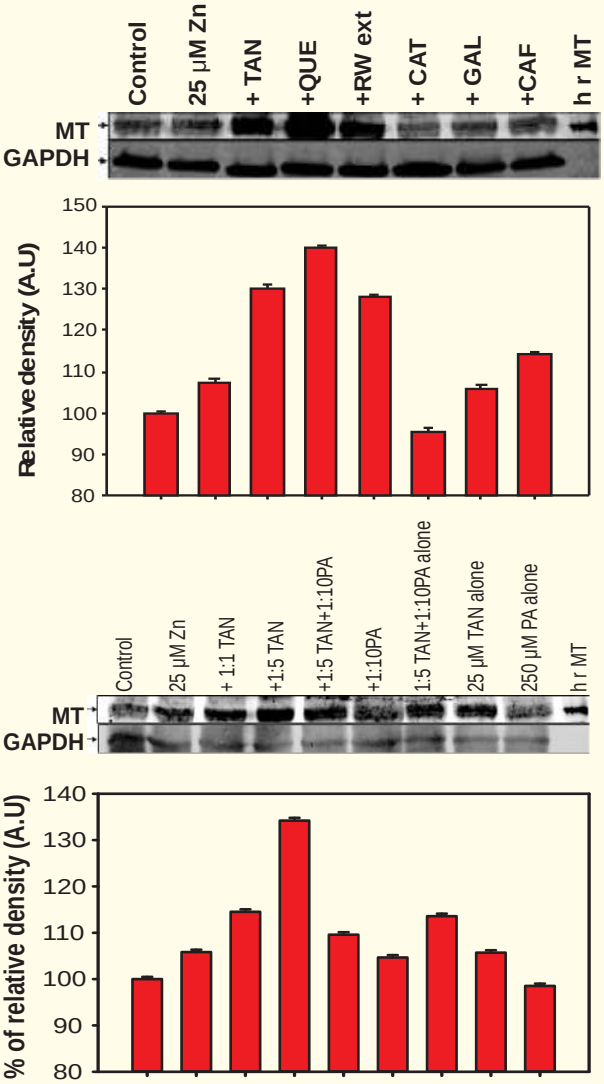


Fig 2. Induction of metallothionein expression by polyphenols: Caco-2 cells were incubated with 25µmole/L zinc either in the presence or absence of polyphenols (A) phytic acid on tannic acid (B). Metallothionein expression was assessed by 12 % SDS-PAGE followed by immunoblotting and detection by biotinylated anti MT IgG and streptavidine-biotin–peroxidase followed by chemiluminiscence method. Bar diagram represents the relative density of band intensity was measured with quantity one software (Bio-Rad). CAT- Catechin, GAL-Gallic acid, CAF-Caffeic acid, TAN- Tannic acid, QUE-Quercitin.

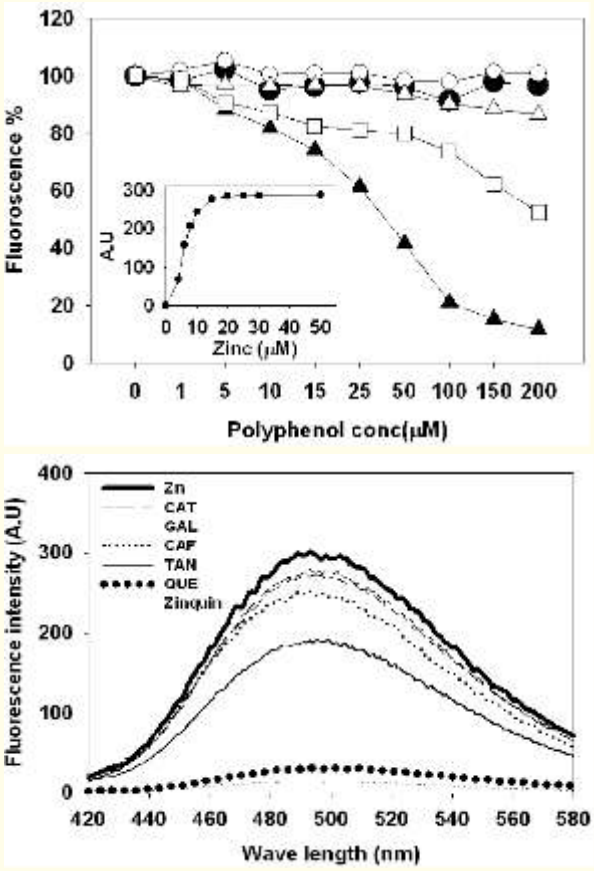


Fig 3. Binding of zinquin, polyphenols with zinc: A; Dose dependent quenching (%) of zinc induced zinquin fluorescence in the presence of tannic acid ( ),quercitin, catechin , gallic acid , and caffeic acid Zinc dependent zinquin fluorescence (Inset A). B; Fluorescence spectra of zinc induced zinquin fluorescence quenching in the presence of (1:20 molar concentrations of zinc: polyphenol) various polyphenols. CAT- Catechin, GAL-Gallic acid, CAF-Caffeic acid, TAN- Tannic acid, QUE-Quercitin.

CONCLUSIONS

These results suggest that polyphenol-rich beverages such as RW, RGJ and GT or certain specific polyphenols (tannic acid and quercitin) enhance the uptake of zinc in Caco-2 cells. However, there appear to be differences in relative binding and thus variation in zinc uptake in the presence of various polyphenols.

5 SIMULTANEOUS DETERMINATION OF BIOCHEMICAL INDICATORS OF MICRONUTRIENT STATUS FROM FINGER PUNCTURE BLOOD SPOT: SIMULTANEOUS DETERMINATION OF RETINOL AND ALPHA-TOCOPHEROL AND INDIVIDUAL METHODS FOR ASCORBIC ACID AND 5-METHYLTETRA HYDROFOLATE IN DBS BY HPLC

Assessment of micronutrient status assumes importance not only in estimating the prevalence of deficiencies but also in program evaluation aimed at controlling micronutrient deficiency. Accurate methods that are suitable for field surveys are lacking. The recently developed dried blood spot (DBS) method for vitamin A estimation is a convenient method for assessing sub-clinical deficiency of vitamin A in large-scale surveys.

OBJECTIVES

The objectives were to develop individual methods for ascorbic acid, 5-methyl tetrahydrofolate (5-MTHF for folic acid), retinol and α-tocopherol and to develop simultaneous methods for their determination in DBS by HPLC.

METHODOLOGY

Preparation of DBS

Blood was collected from human volunteers in potassium EDTA vacuette tubes, mixed and 50 L spotted on to each of the 4 circles of 12.5 mm diameter in a whatman 31 – ETCHER filter paper of size 94 mm X 55 mm. The spots were allowed to air dry for 2-3 h at room temperature in the dark, covered in black paper and stored at – 20°C in a zip-lock plastic cover containing silica. For each volunteer, blood sample as such and plasma was also stored at -20°C under identical conditions. DBS punches were cut out with a calibrated paper punch (6.5 l blood volume per punch of DBS) and processed along with plasma.

Simultaneous determination of retinol and vitamin E in DBS

The method developed for the analysis of DBS retinol by HPLC was standardized for the simultaneous determination of retinol and α-tocopherol. Briefly, the method involves extraction of retinol and α-tocopherol from 1 DBS spot, blood and plasma using 1% ascorbic acid containing 0.1% Triton X – 100 followed by protein precipitation with acetonitrile containing 1% BHT. The samples were then extracted with hexane (1%BHT) and the supernatant loaded on to a cyano column (kromasil cyano, 5, 150 X 4.6 mm, Flexit Jour Laboratories Pvt. Ltd. Pune, India) of an HPLC system (Thermo Finnigan). The elution was carried out at 2 ml / min using the mobile phase hexane: isopropanol (98.5:1.5) and detected at 292 nm (α-tocopherol) and 326 nm (retinol).

Standardization of ascorbic acid in DBS Method

Briefly, 3 punches, and 50l plasma was sonicated in 200 L of phosphate buffer pH 7.2 for 10 min (J.Chromatographic Science 15: 262- 266, 1977 and Clin. Chem. 34/11, 2217-2223, 1988). To this 50 l of 3 % metaphosphoric acid was added and incubated for 30 min at room temperature. An aliquot of the supernatant thus obtained was filtered through 0.22m filter and injected on to a C18 column attached to an Agilent 1100 series HPLC system with flow rate of 1 ml/min in a mobile phase of water: methanol (95:5) containing 1 % KCl and detected at 254 nm.

Standardization of estimation of 5MTHF in DBS

The estimation of 5MTHF by HPLC in DBS was standardized and compared with whole blood 5 MTHF and commercial RIA Kit method. Briefly, three punches were sonicated with 1 % ascorbic acid containing 0.1% Triton

X-100 for 15 min in an ultra-sonication bath. The extract was further incubated for 3 h at 37°C in an inert atmosphere of nitrogen. Proteins in the extracts were precipitated with 60 % perchloric acid, frozen for 10 min and thawed. The supernatant thus obtained was filtered through 0.22m filter and 20 l injected on to a C18 column connected to an Agilent 1100 series HPLC system. The elution was carried out at a flow rate 1 ml/min using the mobile phase 33 mM orthophosphoric acid containing 8 % acetonitrile, pH 2.3 and detector set at excitation of 295 nm and emission 365 nm.

RESULTS

1. Retinol levels were comparable between plasma, blood and DBS while α – tocopherol levels were significantly lower in DBS compared to plasma and blood (Table 1, Fig. 1).
2. Retinol in DBS was stable at – 20° C, RT and at simulated field conditions even after 4 weeks. On the other hand α – tocopherol levels in DBS samples deteriorated to extent of more than 66 % at all the temperatures studied with in a week (Fig. 2)

Fig 1. Simultaneous determination of retinol and a-tocopherol in DBS and plasma by HPLC

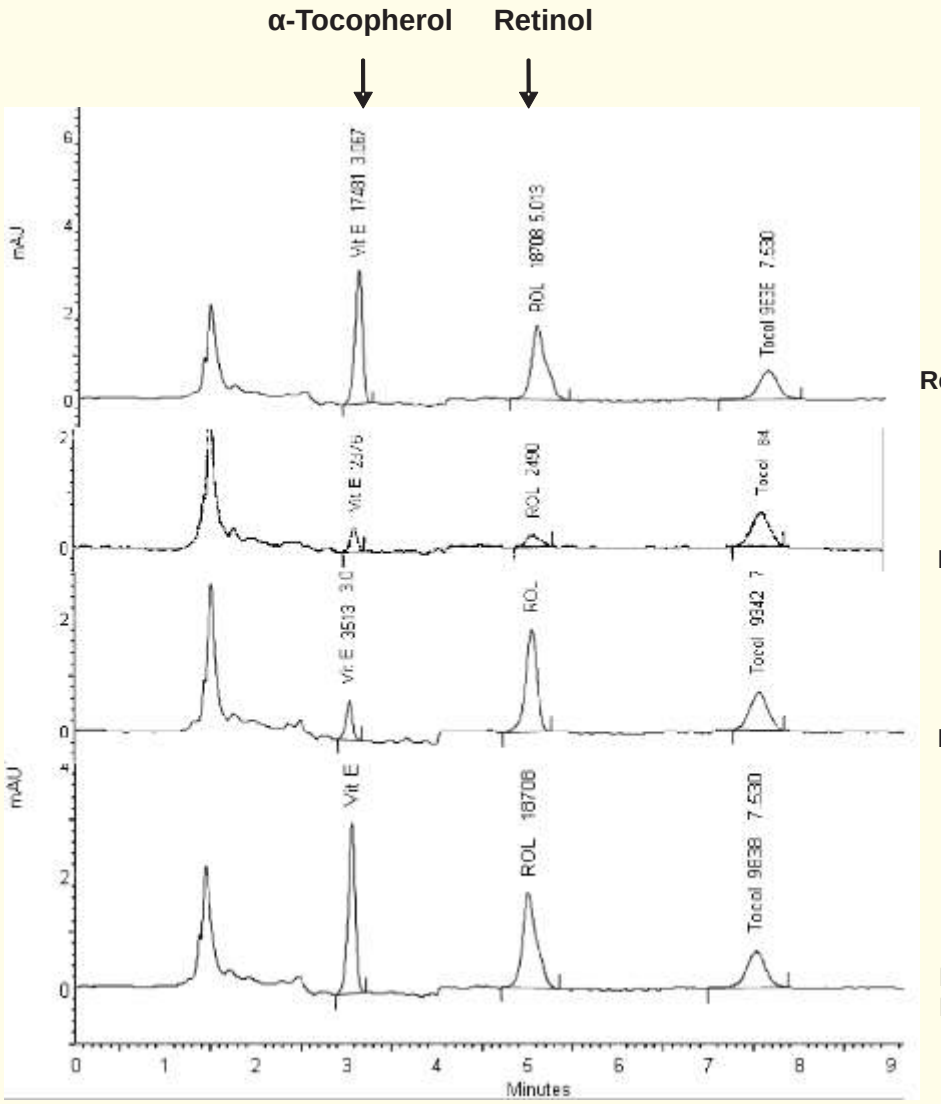


Table 1. Comparison of retinol and α- tocopherol in DBS, blood and plasma

Sample	Retinol (?g/dl)			α-tocopherol (mg/dl)		
	Blood	DBS	Plasma	Blood	DBS	Plasma
Mean ± SD (N= 4)	31.2[a] 9.33	31.5[a] 11.4	30.7[a] 8.31	0.426[a] 0.045	0.275[b] 0.053	0.381[c] 0.036

Means with different superscript letter are significantly different at P < 0.005 by One way ANOVA and post hoc 't'-test

3. For the estimation of ascorbic acid in DBS, 3 DBS punches were required.
4. There were significant differences in the levels of ascorbic acid in plasma compared to DBS and ascorbic acid content in RBC, suggesting that they contribute to the over-estimation of ascorbic acid in DBS (Fig. 3, Table 2)
5. The minimum detection limit of 5MTHF was 20pg.

Table 2. Comparison of ascorbic acid and 5 MTHF levels in DBS and plasma/blood

Parameter	DBS	Plasma/ Blood
Ascorbic acid (mg/dl)	1.86 ± 0.374	1.25 ± 1.198
5MTHF (ng/ml)	228.15± 138.99	239.62 ± 133.05

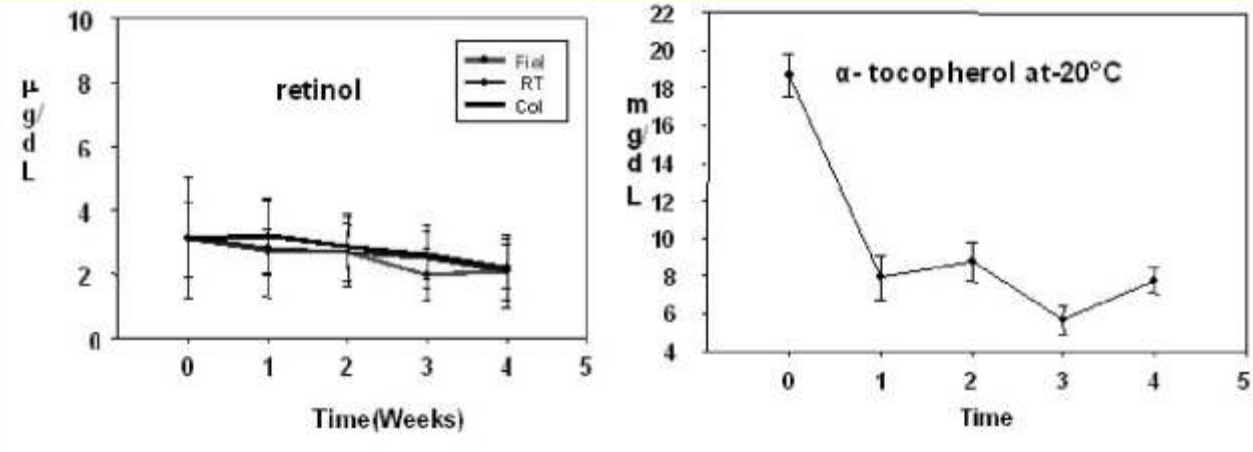


Fig 2. Stability of retinol and -tocopherol in DBS at different storage conditions

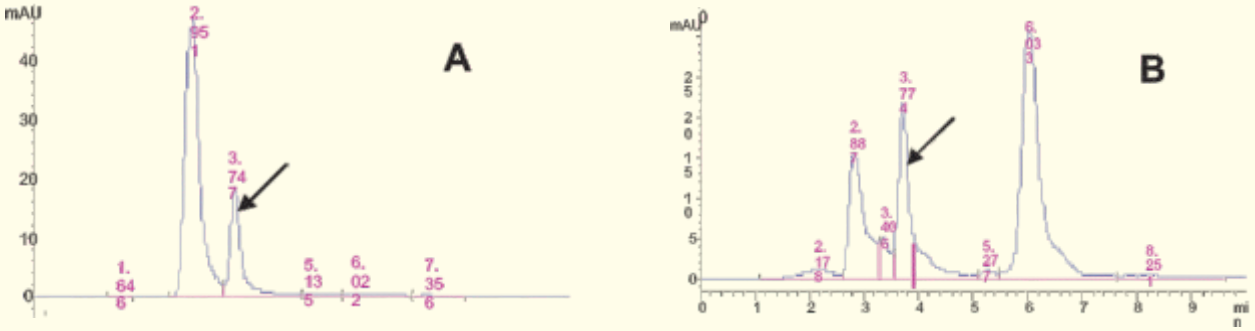


Fig 3. Chromatograms A. standard ascorbic acid. B. DBS sample

Fig 4. Chromatograms of 5MTHF (A), DBS sample (B) and DBS sample spiked with 5MTHF (C)

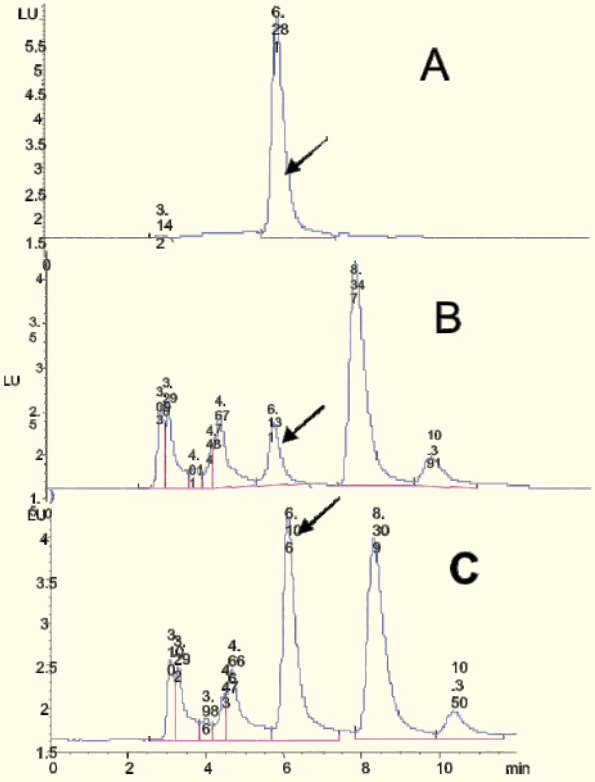
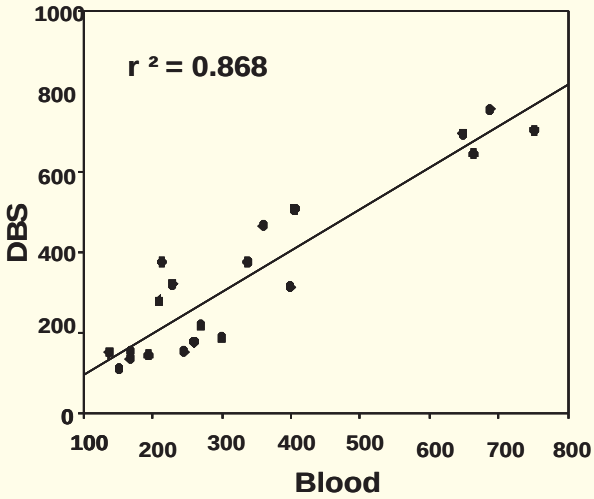


Fig 5. Correlation between 5MTHF in blood and DBS samples



6. Incubation of blood at 37° C for 3 h showed a 3-fold increase in the 5 MTHF levels suggesting actions of endogenous enzymes in converting polyglutamate to 5MTHF. The level of 5MTHF estimated by HPLC in DBS was lower than the folic acid levels estimated by radiometric assay.

7. There was a good correlation (r^2 = 0.8) between the DBS and blood 5MTHF levels. (Fig. 4 & 5). The 5MTHF in normal healthy volunteers ranged between 74 – 289 ng/ml in whole blood.

CONCLUSIONS

Individual methods were developed for estimating ascorbic acid and 5MTHF in DBS. A method for the simultaneous determination of retinol and α – tocopherol in DBS was developed.

6 A STUDY ON PERCEIVED STRESS AMONG HIGHER SECONDARY STUDENTS: IDENTIFICATION OF THE TARGET GROUP

Perceived stress is considered as an outcome variable measuring the experienced level of stress as a function of objective stressful events, coping processes, personality factors etc. Numerous social stressors and high levels of perceived stress have been shown to be positively associated with mortality.

The objectives of the present study were therefore to assess stress perception and coping among the children of Intermediate first year and to assess the gender difference as well as the influence of the type of institution (Government /Private, Single gender/co-education) in stress manifestation & to understand the coping strategies used by them.

METHODOLOGY

The study procedures were approved by the Institutional Review Board of the National Institute of Nutrition, Hyderabad (NIN-IEC/No: 11/07).

Design of the study: A total sample size of 80 was required from six colleges. The study design is given in Scheme -1. Briefly, the colleges in Twin cities (N=256) were categorized into Government (N=18) or Private (N=238) based on the information provided by the Education Department. All of them catered to the low and middle socioeconomic status, providing full–time coaching as per the state syllabus and having at least one division with English as the medium of study. *The stress scales such as perceived stress scale (PSS), general health questionnaire (GHQ), life events scale (LE) and coping scales were administered in Hindi and Telugu.*

The data available for statistical analysis was n=75. Data analysis was done using SPSS version 15.0. The scores on behavioural parameters were categorized by quartile distribution and the coping sub-scales were used for data interpretation on coping strategies. To understand the relation between behavioral parameters Pearson bi-variate correlation was used. To assess the differences in the type of Institution, student 't' test was applied. One-way ANOVA with post hoc analysis was used to compare the differences in stress scores among co-ed and single gender colleges. Gender differences in quartile distribution of stress parameters were assessed by Chi-square test and proportion t test.

RESULTS

Out of 120 students who consented, only 75 completed the study in all respects. The compliance of students was around 63% and satisfied about 94% of the expected sample size of 80.

The background characteristics (age, number of siblings, birth order and family size) showed that the participants were comparable across colleges. All the variables for pooled data positively correlated ($p<0.01$) with each other. The mean age of the students was 16.5 ± 0.97.

The mean score on PSS among the students was 40.48 ± 6.58, and on GHQ it was 8.9 ± 4.16. Life events scale had a mean score of 455.4±176.9. Among the coping subscales, the cognitive and behavioral approach scales showed a mean score of 13.4 ± 3.05 and 30.0 ±7.21 respectively. The cognitive –behavioral

Table 1. Percent distribution of stress among students (pooled data)

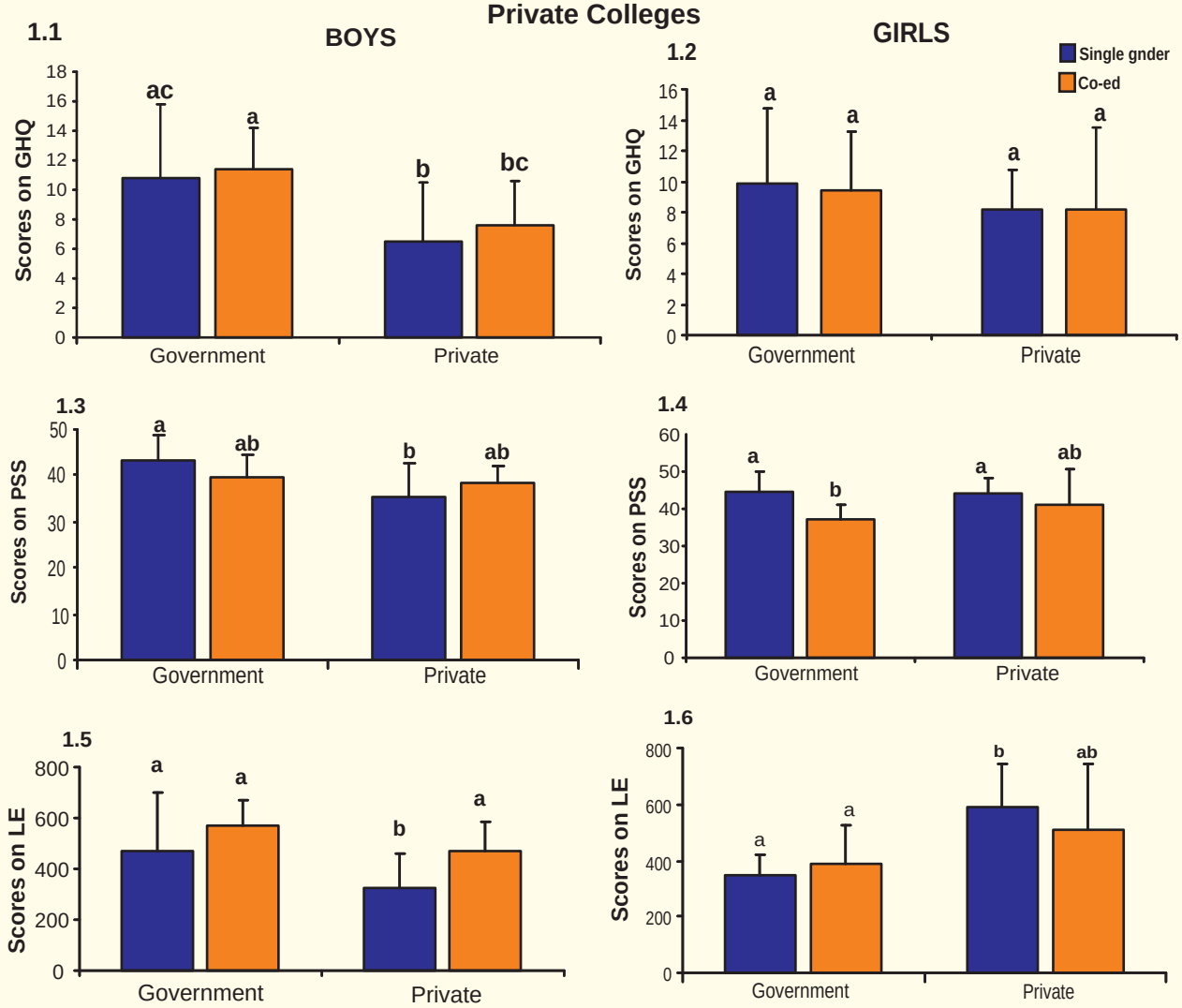
Behavioral parameters	<q1	q1-q2	q2-q3	>q3		
				Pooled	Males	Females
Perceived stress	24.0 (18)	24.0 (18)	26.7 (20)	25.3 (19)	13.2 (5)	37.8* (14)
General health	22.7 (17)	18.7 (14)	30.7 (23)	28.0 (21)	26.3 (10)	29.7 (11)
Coping	20.0 (15)	29.3 (22)	24 (18)	26.7 (20)	28.9 (11)	24.3 (9)
Life events	24.0 (18)	25.3 (19)	25.3 (19)	25.3 (19)	26.3 (10)	24.3 (9)

Figures in parenthesis indicate number of subjects. * P< 0.05 by proportion 't' test.

Table 2: Distribution of stress (with single and 2 parameters) and coping scores based on gender and type of Institution

Gender/College	PSS	GHQ	LE	PSS+ GHQ	Approach	Avoidance
Gender						
Boys (N= 38)	5	10	10	5	35	19
Girls (N=37)	14	11	09	6	32	27
Type of College						
a) Government						
Boys (18)	4	8	7	4	19	16
Girls (18)	6	6	1	4	12	08
b) Private						
Boys (20)	1	2	3	1	16	03
Girls (19)	8	5	8	2	20	19

Figure 1. Mean scores of stress Indicators among Government and Private Colleges



Means with different superscripts are significantly different (p<0.01)

approach mean scores were 19.0± 4.19 .The avoidance scales showed a mean 21.12 ± 7.67 and 13.7 ± 3.91 for behavioral and cognitive avoidance respectively.

Quartile distribution of behavioral stress parameters

The distribution of students in all the four quartiles was similar ie about 25% (Table 1). With more than one behavioral parameter, stress prevalence was only 14% (11/ 75) in PSS-GHQ group, 6.7% in PSS-Low coping, 7% each in PSS-LE and GHQ-LE group and 5.3% in GHQ-low coping group (Table 2). How ever, the percent of students in the highest quartile with three behavioral parameters indicative of stress was about 5.3% and only two students satisfied the criteria for all parameters.

Gender-wise distribution of perceived stress

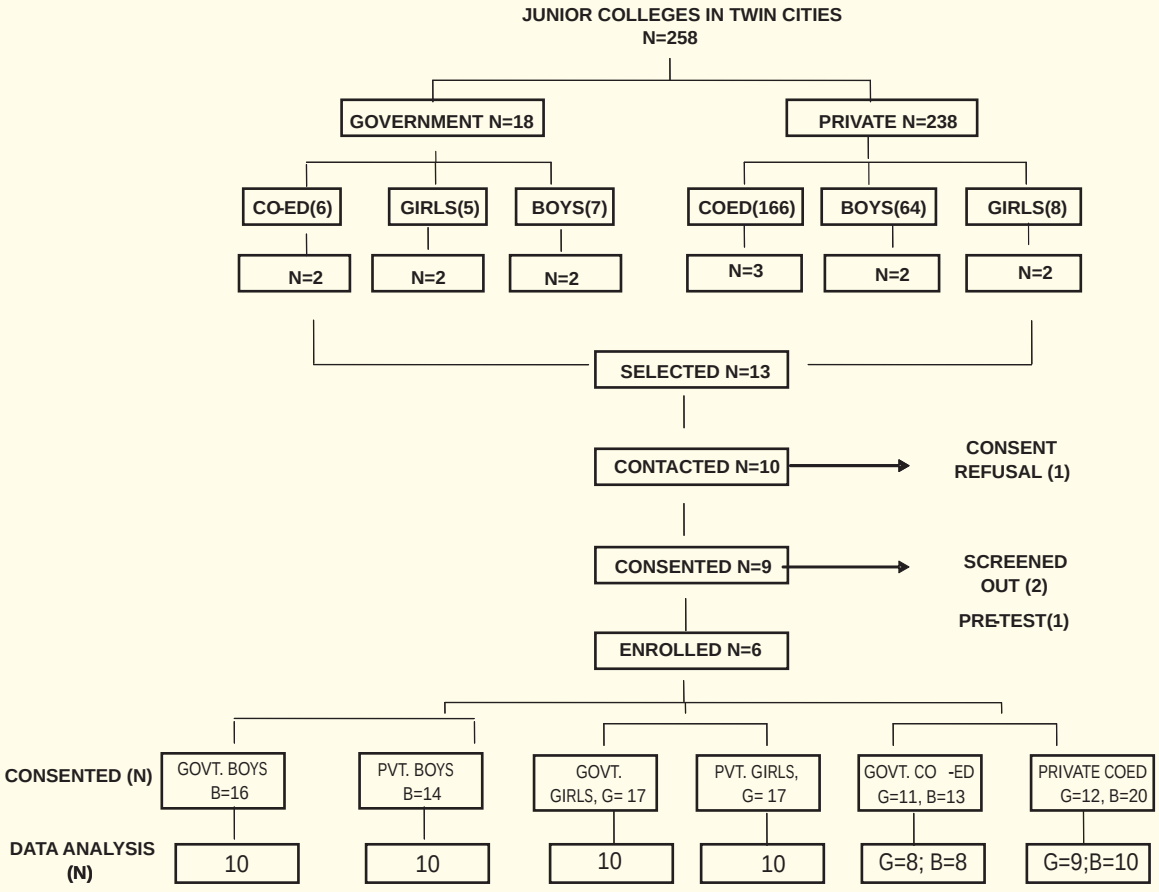
The gender-wise quartile distribution of the behavioral stress parameters showed no significant difference between males and females. However, on proportion 't' test (>q3), girls were found to be significantly different in stress prevalence than boys (Table 1).

Categorization of subjects based on two indicators of perceived stress showed no significant gender difference and identified 7 out of 20 in single gender government colleges and 2 out of 19 in single gender private colleges as stressed.

Type of Institution

Stress manifestation in relation to the type of college showed higher mean scores on

Scheme 1. Study design



GHQ ($p<0.005$) in students belonging to Government (10.2 ± 4.14) compared to students of Private colleges (7.6 ± 3.81). This difference was mainly due to the high score of boys of Government college (Fig. 1.1 & 1.2). Government college boys showed higher scores on PSS than students of Private colleges. Government girls colleges also showed a significant difference from the corresponding co-ed college. Among Private schools, girls and boys of single gender colleges exhibited more perceived stress than co-ed (Panel 1.3 and1.4).

Boys of Government college had a significantly higher scores on LE compared to other categories (Panel1.5). An opposite result was observed in girls (Panel 1.6).

COPING STRATEGIES AMONG STUDENTS

Single gender college students as a whole showed a higher use of emotion focused coping (Table 2) except for Private boys. The Private boys' college students used less of avoidance coping strategies and an overall less manifestation of perceived stress. More female students reported emotion-focused (avoidance) coping strategies than boys. However, all together, the students irrespective of the education system they belong to, were found to be using a mixture of coping strategies and there was no statistically significant difference between any of the groups.

CONCLUSIONS

1. One fourth of the students belonged to the topmost quartile in all the three stress parameters.
2. Girls reported significantly higher scores on proportion't' test in perceived stress scores.
3. Except for life events among Private girls' college students, the mean stress scores were high for students of Government colleges. Pooled data also showed significant difference for GHQ scores
4. Students of single gender colleges had higher perceived stress compared to co-ed colleges.
5. More number of students from Government boys' College showed high stress considering two parameters and hence will be a good target group for further studies.

7 METABOLIC PROGRAMMING OF INSULIN RESISTANCE: ROLE OF MATERNAL AND PERI / POSTNATAL CHROMIUM STATUS IN THE OFFSPRING – ADIPOSITY, GLUCOSE AND LIPID METABOLISM

The fetal origins of adult disease (FOAD) is hypothesized based on the observations that environmental factors redirect the developmental path during the fetal growth and land up finally with obesity, cardiovascular diseases and metabolic disorders. We reported earlier that maternal vitamin and mineral restriction altered body fat content, plasma lipids and oxidative stress in WNIN rat offspring and may predispose them to IR in later life. Chromium, known as glucose tolerance factor, enhances glucose utilization by insulin target tissues in animals and humans. The chromium-binding oligopeptide, chromodulin activates tyrosine kinase activity of the insulin receptor in response to insulin. While marginal Cr deficiency may increase risk for diabetes, Cr deficiency in persons receiving total parenteral nutrition results in glucose intolerance, peripheral neuropathy,

brain dysfunction, gestational diabetes and coronary heart disease. However, the influence of maternal Cr deficiency on the body adiposity, lipid metabolism, IR / impaired glucose metabolism in the offspring is not yet explored. The present study has been conducted to validate / negate the hypothesis that maternal chromium restriction predisposes the offspring to altered body composition and Insulin Resistance (IR) in their later life.

OBJECTIVES

- To assess the effect of maternal, peri and postnatal dietary Cr deficiency on the body composition (adiposity) and IR in the offspring.
- To determine the biochemical and / or molecular changes associated with altered body composition (development and function of adipose tissue), impaired glucose tolerance and insulin resistance.
- To evaluate the prevention / reversibility of the phenotypic and associated biochemical changes in the offspring by Cr rehabilitation of different points of initiation and duration.

EXPERIMENTAL DESIGN

Female, weanling, Wistar NIN (WNIN) rats (n=30) were divided into two groups of 6 and 24. The group of 24 rats was fed for 12 weeks , a casein based (18% protein) Cr restricted diet containing 0.51mg Cr / kg diet. The other group of 6 rats received the same diet with 1.56 mg Cr per kg diet (control) . The animals were fed their respective diets for 12 weeks, their Cr status monitored and mated with control males. As reported earlier (Annual report 2005-2006), dietary Cr restriction for three months had no significant effect on glucose tolerance, insulin resistance, plasma lipid profile or the reproductive performance of WNIN female rats.

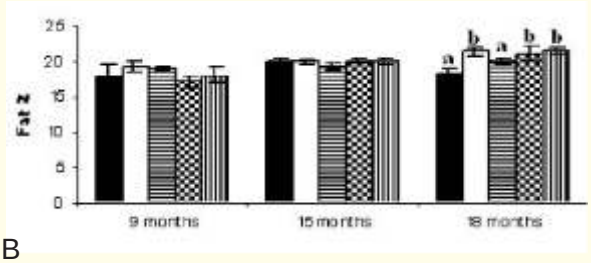
From conception, six pregnant dams from Cr restricted group were switched to control diet (CrRC) while the remaining mothers continued on Cr restricted diet throughout pregnancy. At parturition, six mothers from Cr restricted group were switched to control diet (CrRP) and the remaining Cr restricted mothers continued on restricted diet till weaning. At weaning, half the number of Cr restricted offspring were weaned onto control diet (CrRW), while the remaining offspring continued on restricted diet (CrR). At every 3 months time interval, body composition (by TOBEC) , biochemical indices related to glucose tolerance and insulin resistance and plasma lipid profile were monitored in both male and female offspring of all the groups and the salient findings are as follows.

Body fat %:

Males

Compared to CrC, body fat % was significantly higher (Fig 1A) in CrR offspring at 18 months of age but not earlier. Further, CrRP but not CrRW appeared to correct this insult whereas CrRC had no effect on this parameter.

A



B

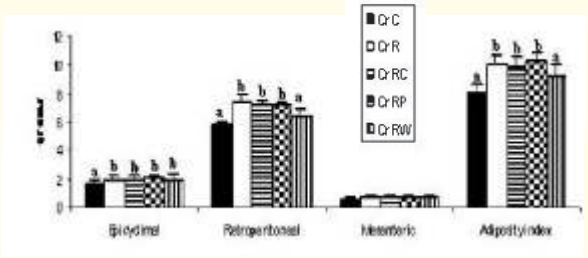


Fig 1. Fat % (A), wet weights of fat deposits and adiposity index (B) of male offspring Effect of maternal Cr restriction and rehabilitation on fat% and adiposity. Panel A: fat%, n=6 of different groups at different ages, panel B: wet weights of three fat deposits and

adiposity index, n=6 of different groups at 18 months of age. Values are mean ± SE. Bars without a common superscript are significantly different at p< 0.05 by one way ANOVA.

Females

CrR had significantly higher (p<0.05) body fat % (than CrC) as early as 3 months of their age and this continued till their sacrifice at 15 months of age (Fig 2A). While CrRC could correct the change at all time points studied, CrRP and CrRW, could not do so , specially at 15 months of age.

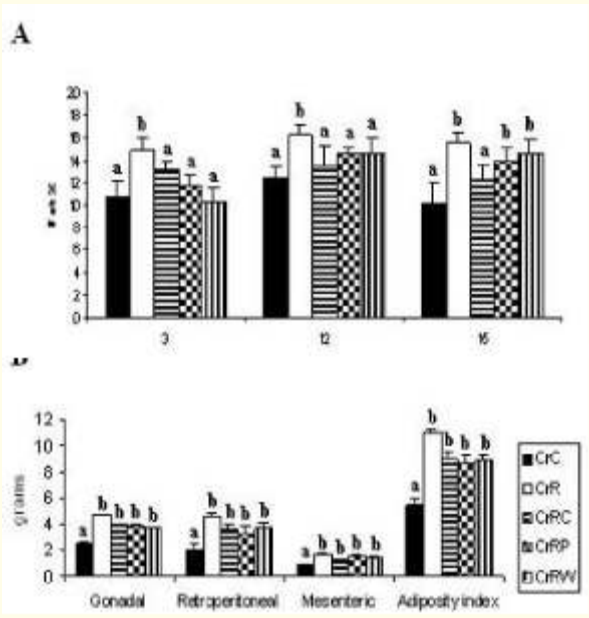


Fig 2. Fat % (A), wet weights of fat deposits and adiposity index (B) of female offspring Effect of maternal Cr restriction and rehabilitation on fat% and adiposity. Panel A: fat%, n=6 of different groups at different ages, panel B: wet weights of three fat deposits and adiposity index, n=6 of different groups at 15 months of age. Values are mean ± SE. Bars without a common superscript are significantly different at p< 0.05 by one way ANOVA.

These observations suggest that maternal Cr restriction significantly increased body fat % in CrR offspring compared to controls in both male and female offspring. Unlike males where the effect was seen only at 18 months of age but not earlier, females developed the insult as early as 3 months and continued till the time of their sacrifice.

To decipher whether the increased body fat % developed in CrR offspring was associated with increased central / visceral adiposity, adiposity index, a marker for visceral adiposity was monitored.

ADIPOSITY INDEX

The wet weights of the three visceral fat deposits: epididamal, mesenteric and retroperitoneal fat pads were determined and adiposity index was computed in the offspring of different groups. It was observed that the wet weights of the three fat pads were significantly higher in CrR offspring in both the genders and none of the rehabilitation regimes could correct the changes (Fig 1B & 2B).

Expression of genes involved in adipogenesis

To assess whether the changes observed in the body fat and central adiposity were associated with changes in gene expression at transcriptional level, expression of genes involved in adipogenesis / adiposity was monitored in the adipose tissue.

It was noticed that the expression of PPAR, SREBP, adiponectin and Fas which are involved in adipogenesis / adiposity and lipid synthesis were comparable among the offspring of different groups in both the genders (Fig 3 & 4). It thus appears that the effects of maternal Cr restriction on adipogenesis and lipid metabolism may not be associated with changes in gene expression at transcription level.

PLASMA LIPID PROFILE

To assess whether altered body adiposity was associated with impaired lipid metabolism as well as to investigate whether maternal Cr restriction affected lipid metabolism, the plasma lipid profile was determined in the offspring.

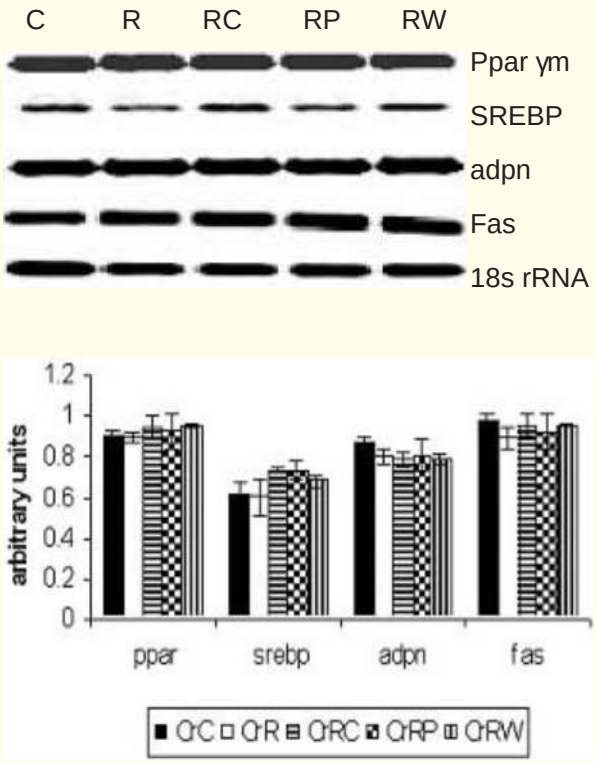


Fig 3. Effect of maternal Cr restriction and rehabilitation on adipogenesis / adiposity of male offspring. Expression of genes involved in adipogenesis / adiposity by semi-quantitative PCR in adipose tissue (n=3) at 18 months of age. Values are mean ± SE.

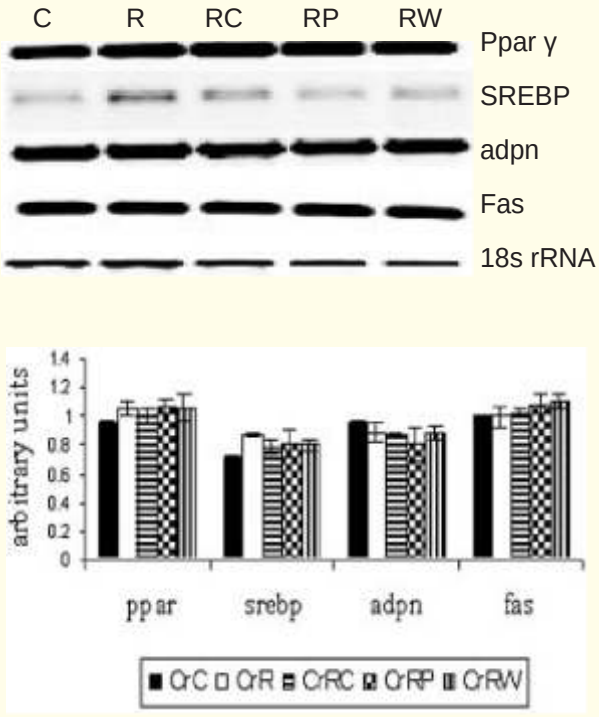


Fig 4. Effect of maternal Cr restriction and rehabilitation on adipogenesis / adiposity of female offspring. Expression of genes involved in adipogenesis / adiposity by semi-quantitative PCR in adipose tissue (n=3) at 15 months of age. Values are mean ± SE.

Males

Plasma triglycerides, total cholesterol, HDL cholesterol and free fatty acids were comparable among the groups at all the time points tested. The little changes seen in CrR offspring appeared to be corrected by all the rehabilitation regimes in general (Fig 5).

Females

Plasma triglycerides and free fatty acids were significantly higher ($p<0.05$) in CrR than CrC offspring from 9 months of their age and all three rehabilitation regimens mitigated the change (Fig 6C & 6D). However, plasma total cholesterol and HDL cholesterol were comparable among the groups through out their life (Fig 6A & 6B).

ADIPOSE TISSUE FUNCTION

Considering that some adipocytokines like leptin, adiponectin, TNF, Il-1, Il-6, MCP-1, PAI influence the manifestation of adiposity / insulin resistance and to check whether maternal Cr restriction induced changes on the adipose development and function in the offspring were mediated through changes in their expression, the levels of these adipocytokines were determined in circulation as well as in the adipose tissue.

ADIPOCYTOKINES IN PLASMA

Males

Plasma levels of adiponectin, leptin, IL-6, MCP-1, PAI (active) were comparable among the groups. Interestingly TNF levels were significantly higher in CrR than CrC group and

the three rehabilitation regimens could correct the insult. IL-1 levels were significantly lower in CrR than CrC offspring but no rehabilitation regime could correct the change. (Table 1).

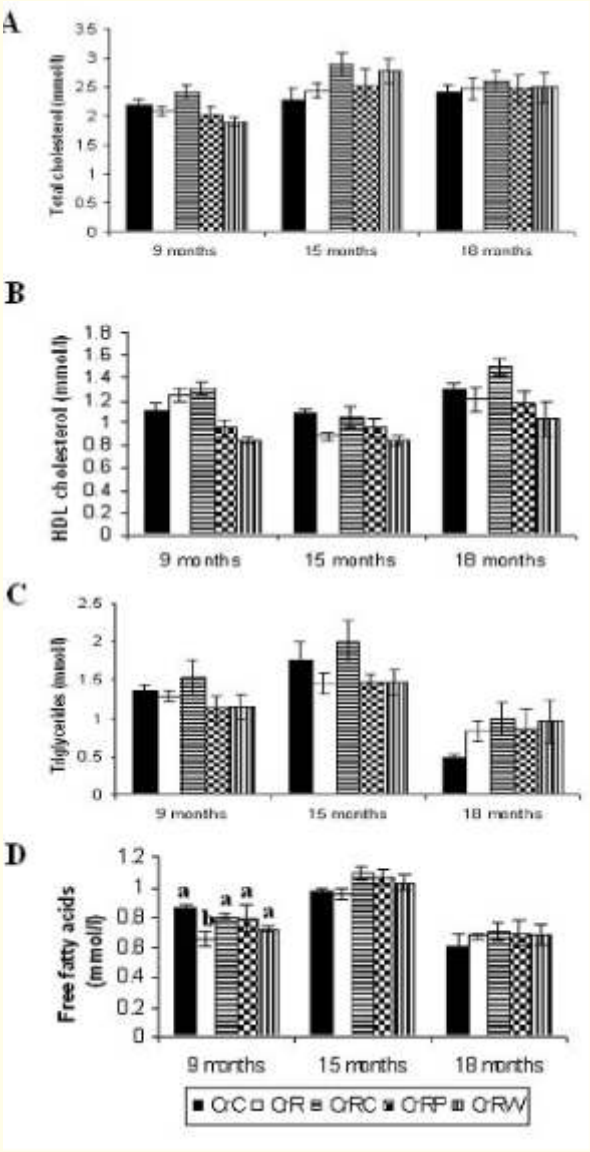


Fig 5. Effect of maternal Cr restriction and rehabilitation on plasma lipid profile of male offspring. Panel A: Total cholesterol, n=6, panel B: HDL cholesterol, n=6, panel C: Triglycerides, n=6 and panel D: Free fatty acids, n = 6 of different groups at different ages. Values are mean ± SE. Bars without a common superscript are significantly different at $p< 0.05$ by one way ANOVA.

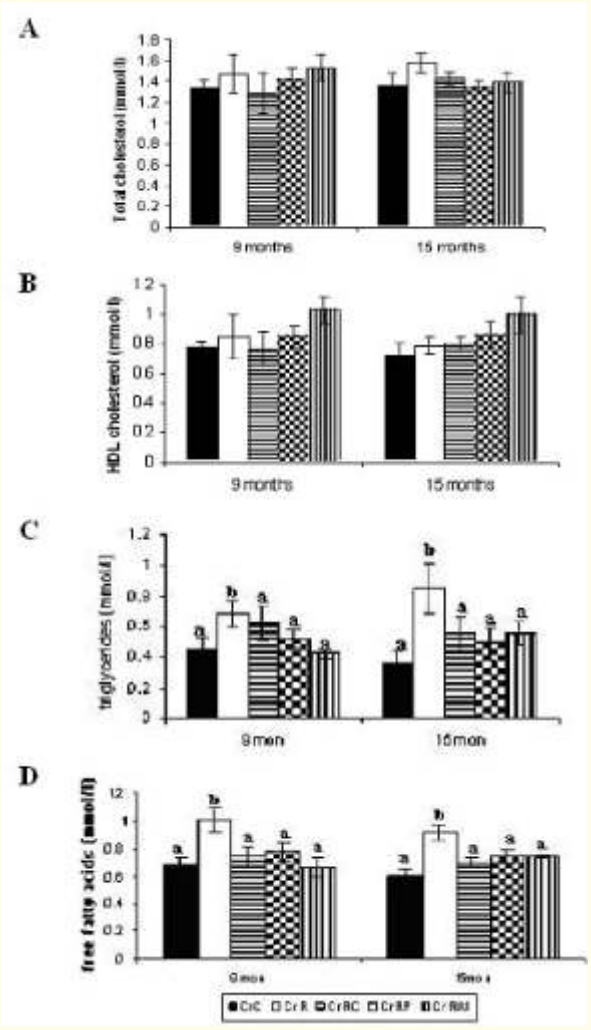


Fig 6. Effect of maternal Cr restriction and rehabilitation on plasma lipid profile of female offspring. Panel A: Total cholesterol, n=6, panel B: HDL cholesterol, n=6, panel C: Triglycerides, n=6 and panel D: Free fatty acids, n = 6 of different groups at different ages. Values are mean ± SE. Bars without a common superscript are significantly different at $p< 0.05$ by one way ANOVA.

Females

Unlike males, leptin and TNF levels were significantly higher in CrR female offspring than CrC . IL-1 and MCP-1 levels were significantly lower in CrR than CrC, whereas adiponectin, IL-6 and PAI (active) were comparable. In general, all rehabilitation

regimes corrected the changes albeit partially (Table 1).

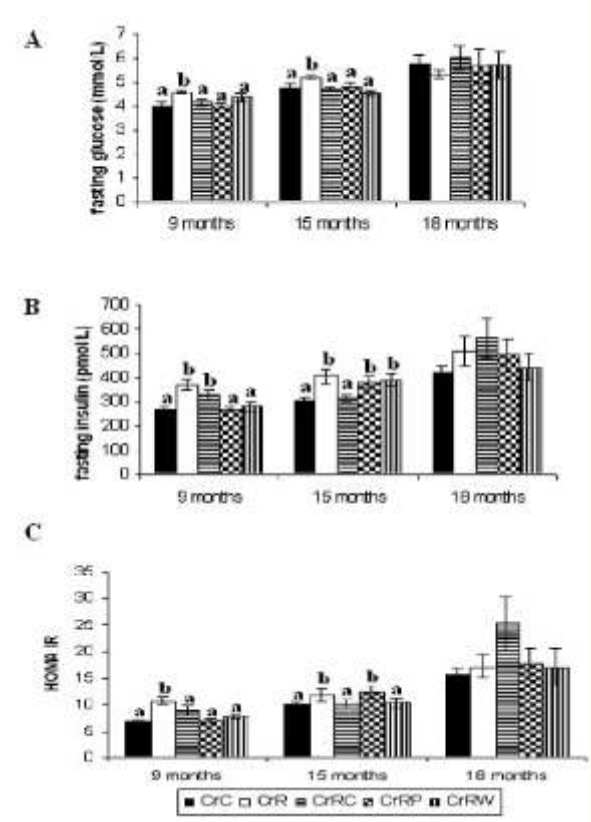


Fig 7. Effect of maternal Cr restriction and rehabilitation on glucose tolerance and insulin response of male offspring. Panel A: fasting glucose, n=6, panel B: fasting insulin, n=6 and panel C: HOMA-IR, n=6 of different groups at different ages. Values are mean ± SE. Bars without a common superscript are significantly different at $p< 0.05$ by one way ANOVA.

Taken together with increased body fat % and central adiposity, increased expression of adipocytokines (leptin, TNF-) in CrR offspring and its partial reversibility by rehabilitation indicates that maternal Cr restriction affects the development and function of adipose tissue in the offspring.

ADIPOCYTOKINES IN ADIPOSE TISSUE

To evaluate whether the changes seen in the circulating levels of leptin, TNF- and IL-1 in the CrR offspring were associated with similar changes in the adipose tissue, these adipokines were quantified in adipose tissue lysate using Lincoplex research kits.

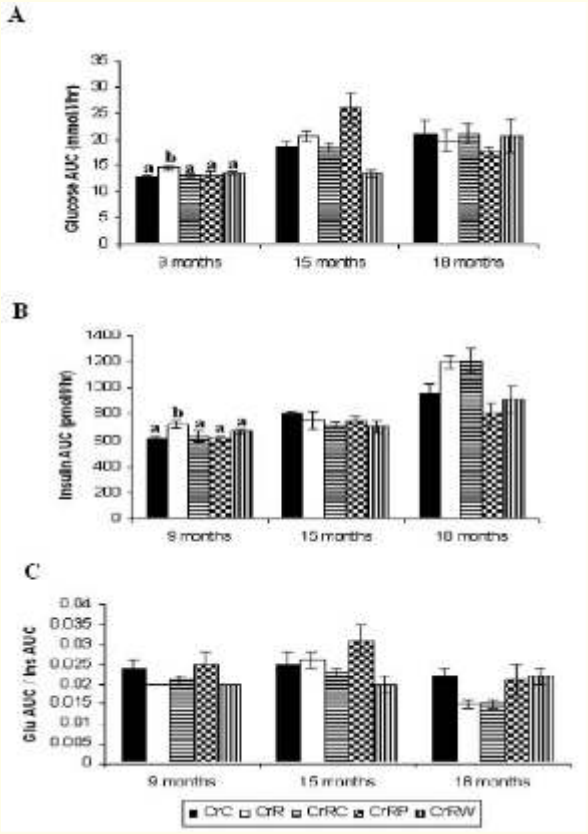


Fig 8. Effect of maternal Cr restriction and rehabilitation on glucose tolerance and insulin response of male offspring. Panel A: AUC glucose, n=6, panel B: AUC insulin, n=6 and panel C: AUC glucose / AUC insulin, n=6 of different groups at different ages. Values are mean ± SE. Bars without a common superscript are significantly different at $p< 0.05$ by one way ANOVA

Males

Levels of Adiponectin and IL-1 were lower whereas PAI (active) was higher in CrR than CrC offspring. CrRC and CrRP did not correct adiponectin levels, whereas IL-1 levels were restored to control levels by CrRC and CrRW but not CrRP. That CrRW significantly increased PAI (active) levels probably suggests the importance of Cr during lactation in modulating the expression of this

adipocytokine.. The levels of other cytokines i.e. leptin, TNF, IL-6 and MCP-1 were comparable among groups (Table 2).

Females

Unlike the male offspring, levels of all the adipocytokines studied in the adipose tissue in female offspring were similar and comparable among different groups (Table 2).

The discrepancy in the levels of different adipocytokines in circulation and adipose tissue in female but not male offspring are somewhat puzzling. They probably suggest differences between the genders in the synthesis (in adipose tissue) and secretion (in to circulation) of adipocytokines and / or differences between genders in the effects of maternal Cr restriction in this process. Clearly, further in depth investigations are needed to clarify these discordant observations.

INSULIN RESISTANCE, GLUCOSE TOLERANCE AND INSULIN RESPONSE TO GLUCOSE CHALLENGE

Males

There were no significant differences among the offspring of different groups in these parameters till postnatal day 180. However at 9 and 15 months of age CrR offspring had significantly higher ($p<0.05$) fasting glucose and insulin levels than CrC . While all three rehabilitation regimes corrected the fasting hyperglycemia (Figure 7A), CrRC but not CrRP or CrRW mitigated the fasting hyper insulinemia at 15 months of age (Figure 7B). As a result CrR offspring had significantly higher ($p<0.05$) HOMA-IR values than CrC offspring at 9 and 15 months of age and were corrected in general by all three rehabilitation regimes (Figure 7C).

Similar to fasting glucose and insulin, glucose AUC & insulin AUC during OGTT were higher in CrR than CrC offspring. However the differences were significant only at 9 months of age but not earlier or later, indicating the transient nature of the change (Figure 8A & 8B). Also, the ratio of glucose AUC / insulin AUC was not different among groups at any time point (Figure 8C). Interestingly, all three rehabilitation regimes appeared to mitigate these changes. However it was surprising that some of the changes observed at 9 and 15 months of age were not seen at 18 months perhaps reiterating the transient nature of the changes.

Females

Fasting plasma glucose was significantly higher ($p<0.05$) in CrR than CrC at 9 and 15 months of age. CrRC but not CrRP or CrRW appeared to mitigate the changes at 9 months of age, while all three regimens corrected the change by 15 months of age (Figure 9A). Fasting plasma insulin levels were comparable among groups till 12 months of age but at 15 months, CrR had significantly higher ($p<0.05$) levels than CrC and none of the rehabilitation regimes could mitigate the change (Figure 9B).

In line with these observations, HOMA-IR was significantly higher ($p<0.05$) in CrR than CrC at 9 and 15 months of age. While all rehabilitation regimes corrected the change albeit partially at 9 but not at 15 months of age (Figure 9C), indicating higher insulin resistance in CrR offspring than CrC and its irreversibility by rehabilitation.

Glucose AUC was comparable among groups at 9 months, but at 15months it was significantly higher ($p<0.05$) in CrR than CrC offspring and all three rehabilitation regimes mitigated the change (Figure 10A). Insulin AUC was also significantly higher ($p<0.05$) in CrR at 15months of age but not earlier. Surprisingly, CrRP and CrRW but not CrRC appeared to correct these changes *albeit* partially (Figure 10B). The ratio of AUC glucose/AUC insulin was significantly decreased ($p<0.05$) in CrR offspring at 15 months of age but not earlier and all the rehabilitation regimes mitigated the change (Figure 10C).

EXPRESSION OF GENES INVOLVED IN GLUCOSE AND LIPID METABOLISM

Phosphoenol pyruvate carboxy kinase (PEPCK), Glucose 6 phosphatase and glycogen synthase are the rate limiting enzymes involved in gluconeogenesis and glycogen synthesis pathways respectively. Expression of these genes involved in glucose metabolism and that of fas gene involved in lipid metabolism were determined in liver by semi quantitative PCR.

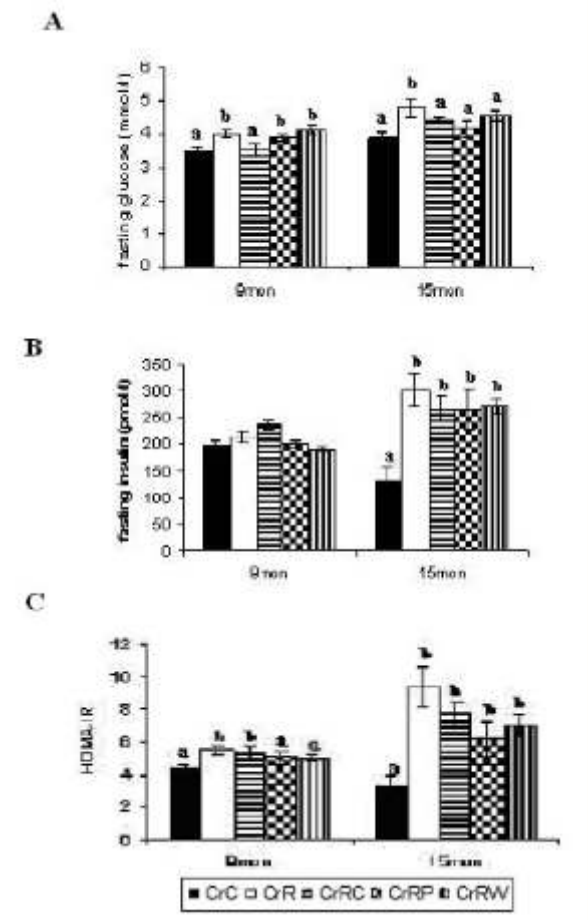


Fig 9. Effect of maternal Cr restriction and rehabilitation on glucose tolerance and insulin response of female offspring. Panel A: fasting glucose, n=6, panel B: fasting insulin, n=6 and panel C: HOMA-IR, n=6 of different groups at different ages. Values are mean ± SE. Bars without a common superscript are significantly different at $p < 0.05$ by one way ANOVA.

Expression of PEPCK, glucose 6 phosphatase, glycogen synthase and fatty acid synthase were comparable among the offspring (male and female) of different groups (Fig 11 & 12). These observations indicate that maternal Cr restriction induced changes in the glucose and lipid metabolism(s) in the offspring may not be due to / associated with transcriptional level changes in the expression of genes of rate limiting enzymes of glucose metabolism and lipid synthesis.

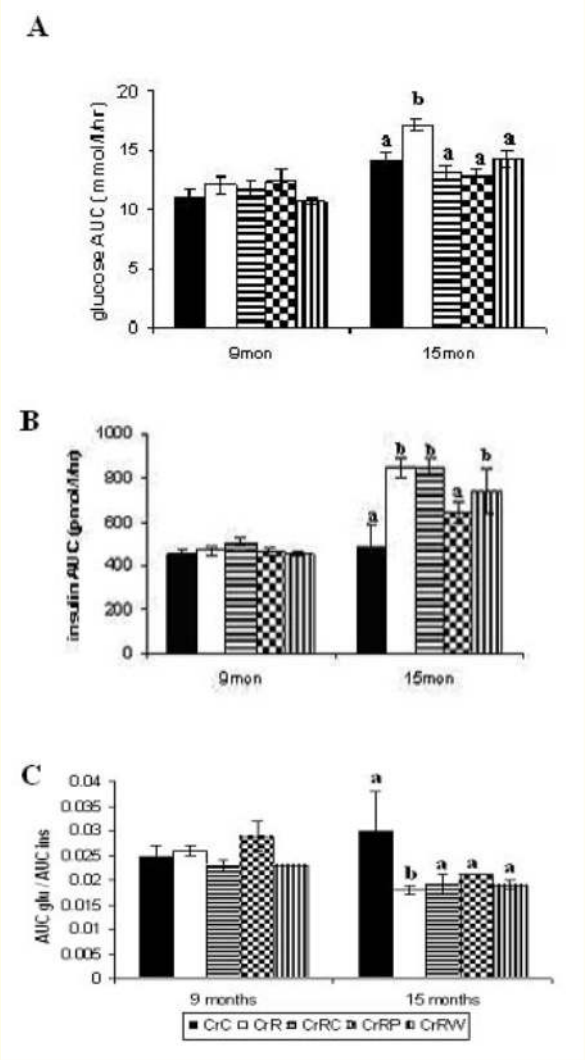
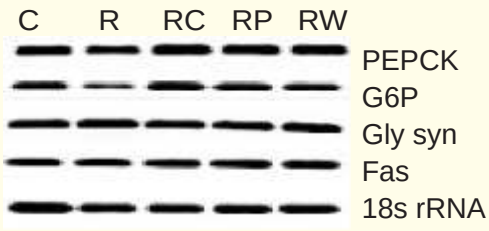


Fig 10. Effect of maternal Cr restriction and rehabilitation on glucose tolerance and insulin response of female offspring. Panel A: AUC glucose, n=6, panel B: AUC insulin, n=6 and panel C: AUC glucose / AUC insulin, n=6 of

different groups at different ages. Values are mean ± SE. Bars without a common superscript are significantly different at p< 0.05 by one way ANOVA.

EXPRESSION OF GENES INVOLVED IN INSULIN SYNTHESIS IN PANCREAS

The two-gene system of insulin in rats and mouse is composed of pre-proinsulin 2 (Ins2), an ortholog of the insulin genes in other mammals and pre-proinsulin (Ins1), a rodent-specific retrogene. Ins2 and Ins1 are both expressed in the pancreas and encode proinsulin peptides.



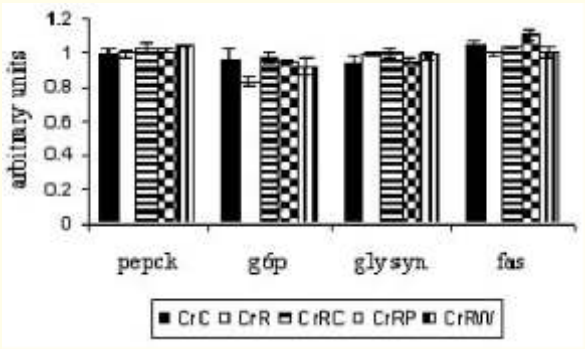
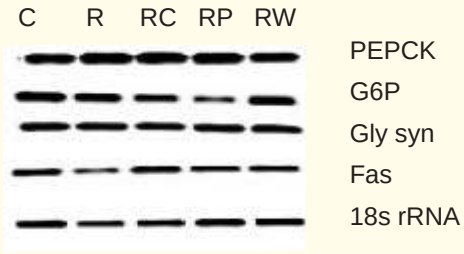


Fig 11. Effect of maternal Cr restriction and rehabilitation on glucose and lipid metabolism of male offspring. Expression of genes involved in gluconeogenesis, glycogen synthesis and lipid synthesis by semi-quantitative PCR in liver tissue (n=3) at 18 months of age. Values are mean ± SE.

Males

Expression of Ins 1 and Ins 2 genes was significantly increased in the pancreas of male CrR offspring. While none of the rehabilitation regimes corrected the change in ins1 gene expression, CrRP and CrRW but not CrRC corrected the change in the ins 2 gene expression (Fig 13).



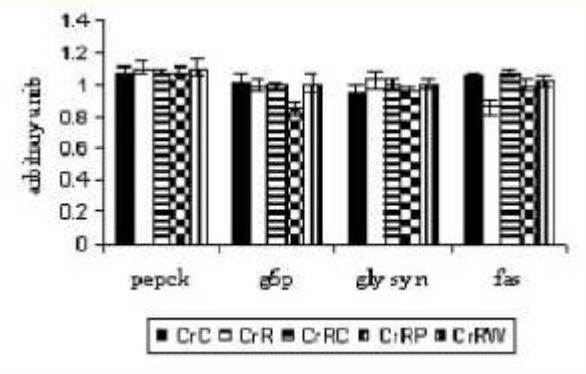


Fig 12. Effect of maternal Cr restriction and rehabilitation on glucose and lipid metabolism of female offspring. Expression of genes involved in gluconeogenesis, glycogen synthesis and lipid synthesis by semi-quantitative PCR in liver tissue (n=3) at 15 months of age. Values are mean ± SE.

Females

Unlike males, expression of ins 1 gene was comparable among different groups of female offspring while ins 2 gene expression was significantly lower ($p<0.05$) in CrR than CrC offspring and all three rehabilitation regimes corrected the change.

These findings indicate that maternal Cr restriction altered the expression of genes involved in insulin synthesis and to the best of our knowledge this is the first report showing changes in pancreatic insulin gene(s) expression and suggests the importance of maternal Cr status in modulating insulin synthesis in the offspring.

Probable mechanism of the effect of maternal Cr restriction

Considering the importance of 11 β HSD1 in modulating adiposity, specially the central adiposity, insulin resistance and metabolic syndrome and that there was a significant and irreversible increase in central adiposity in CrR offspring, we assessed next whether this was associated with increased expression of 11 β HSD1 in the offspring of different groups.

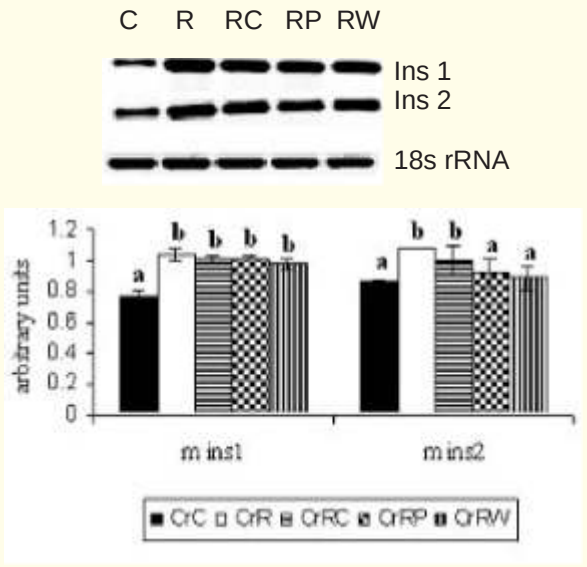


Fig 13. Effect of maternal Cr restriction and rehabilitation on insulin synthesis. Expression of genes involved in insulin synthesis by semi-quantitative PCR in pancreatic tissue in male offspring (n=3, 18 months) Values are mean ± SE. Means without a common superscript are significantly different at p< 0.05 by one way ANOVA.

Expression of 11 β HSD1 in liver

Expression of 11 HSD1 in male offspring was comparable between CrR and control groups and surprisingly the expression was significantly decreased in the offspring of all rehabilitation groups. Unlike males, it was curious that the expression of this gene was down regulated in CrR female offspring. However, all the three rehabilitation regimes corrected the change (Fig 14).

OXIDATIVE STRESS

Males

Chronic maternal chromium restriction resulted in a significant increase ($p<0.05$) in MDA levels in CrR offspring and CrRC and CrRP but not CrRW restored this change (Table 3). On the other hand protein carbonyl and glutathione levels (reduced & oxidized) were comparable among different groups and so was the catalase activity. Interestingly, activities of SOD and GPx were significantly reduced ($p<0.05$) in CrR offspring (Table 3). It was yet surprising that CrRP and CrRW but not CrRC corrected the change in SOD activity whereas the three rehabilitation regimes corrected the insult in GPx activity.

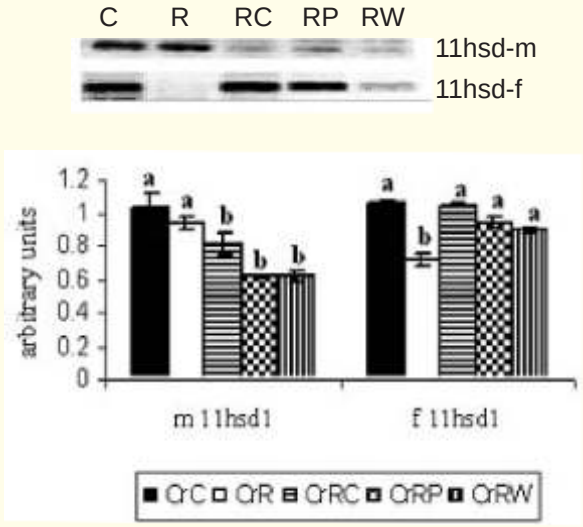


Fig 14. Effect of maternal Cr restriction and rehabilitation on the expression of 11hsd1 by semi-quantitative PCR in liver tissue in both male and female offspring (n=3) at the time of their sacrifice. Values are mean ± SE. Means without a common superscript are significantly different at p< 0.05 by one way ANOVA.

Females

Maternal chromium restriction had comparable effects on lipid peroxides (MDA), protein carbonyls and glutathione (reduced &

oxdised) levels among offspring of different groups. However, the activities of catalase, SOD and GPx were significantly reduced in CrR group compared to CrC. Unlike males, it was interesting that none of the rehabilitation regimes could correct the decrease in SOD and GPx activities, whereas CrRC & CrRP but not CrRW restored the change in catalase activity (Table 4).

Table 1. Plasma adipocytokine levels of different groups of male & female offspring

		CrC	CrR	CrRC	CrRP	CrRW
Adiponectin (?g/ml)	**M**	31.5 ± 3.00	31.5 ± 3.90	33.8 ± 5.31	35.4 ± 8.48	36.2 ± 3.18
	F	17.8± 2.84 [a]	17.5± 3.00 [a]	30.2± 1.05 [b]	27.3± 3.59 [b]	13.2± 2.22 [a]
Leptin (ng/ml)	**M**	4.22 ± 0.407	4.24 ± 0.809	4.57 ± 0.582	2.73 ± 0.685	5.17 ± 0.676
	F	1.11± 0.241 [a]	2.95± 0.318 [b]	1.71± 0.259 [a]	1.46± 0.313 [a]	1.89± 0.199 [a]
TNF-? (pg/ml)	**M**	0.820 ± 0.150[a]	2.28 ± 0.657 [b]	1.46 ± 0.367 [a]	1.34 ± 0.329 [a]	0.905 ± 0.221[a]
	F	1.04± 0.174 [a]	2.59± 0.669 [b]	1.67± 0.392 [a]	1.98± 0.364 [a]	0.996± 0.106[a]
IL-1? (ng/ml)	**M**	0.066 ± 0.012[a]	0.016 ± 0.004[b]	0.033 ± 0.009[b]	0.026 ± 0.004[b]	0.039 ± 0.009[b]
	F	0.102± 0.011[a]	0.044± 0.006 [b]	0.050± 0.005[b]	0.071± 0.020[a]	0.047± 0.010 [b]
IL-6 (ng/ml)	**M**	0.014 ± 0.007	0.018 ± 0.006	0.015 ± 0.007	0.015 ± 0.000	0.015 ± 0.004
	F	0.304± 0.059	0.246± 0.008	0.255± 0.008	0.265± 0.019	0.281± 0.020
MCP-1 (ng/ml)	**M**	0.123 ± 0.025	0.121 ± 0.018	0.070 ± 0.017	0.069 ± 0.024	0.077 ± 0.016
	F	0.246± 0.035 [a]	0.074± 0.022 [b]	0.258± 0.070[a]	0.152± 0.013 [a]	0.198± 0.042 [a]
PAI (ng/ml)	**M**	0.653 ± 0.030	0.762 ± 0.137	0.948± 0.374	0.291 ± 0.133	0.597 ± 0.112
	F	0.417± 0.092	0.500± 0.128	0.541± 0.115	0.476± 0.201	1.02± 0.316
Insulin (ng/ml)	**M**	0.491 ± 0.102	0.484 ± 0.100	0.532 ± 0.089	0.562 ± 0.163	0.424 ± 0.096
	F	0.351± 0.162 [a]	0.927± 0.255 [b]	0.450± 0.108[a]	0.344± 0.076 [a]	0.799± 0.176 [a]

Values are mean SE (n=6)
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA

Table 2. Tissue adipocytokine levels of different groups of male & female offspring

		CrC	CrR	CrRC	CrRP	CrRW
Adiponectin (?g/mg)	**M**	8.56±0.865[a]	5.94±0.425[b]	5.89±0.637[b]	5.71±0.958[b]	6.58±0.492 [a]
	F	5.97±0.434	4.71±.664	5.77±0.317	6.16±0.762	5.25±0.642
Leptin (ng/mg)	**M**	5.12±0.972	5.48±1.03	3.51±0.195	4.82±0.703	4.19±0.585
	F	2.75±0.844	3.05±0.590	2.32±0.467	2.34±0.945	1.87±0.132
TNF-? (pg/mg)	**M**	0.511±0.048	0.602±0.070	0.568±0.040	0.531±0.110	0.606±0.113
	F	0.628±0.119	0.799±0.115	0.718±0.289	0.587±0.085	0.670±0.075
IL-1? (pg/mg)	**M**	5.69±0.794[a]	4.31±0.326[b]	5.62±0.892[a]	4.02±0.436[b]	4.77±0.240 [a]
	F	4.85±0.692[a]	5.44±0.548[a]	2.72±0.601[b]	3.80±0.800[a]	5.23±0.885 [a]
IL-6 (ng/mg)	**M**	0.073±0.008	0.129±0.031	0.120±0.032	0.149±0.069	0.122±0.031
	F	0.200±0.035	0.185±0.062	0.120±0.021	0.212±0.052	0.114±0.039
MCP-1 (ng/mg)	**M**	0.274±0.074	0.168±0.027	0.208±0.024	0.305±0.039	0.203±0.012
	F	0.214±0.034[a]	0.301±0.068[a]	0.189±0.038[a]	0.069±0.015[b]	0.162±0.037[a]
PAI (ng/mg)	**M**	0.291±0.050[a]	1.33±0.154 [b]	0.582±0.076[a]	0.480±0.171[a]	1.07±0.239 [b]
	F	0.428±0.078	0.561±0.208	0.381±0.060	0.659±0.333	0.503±0.170

Values are mean SE (n=6)
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA

Table 3. Oxidative stress and antioxidant status in liver homogenate of different groups in male offspring

	CrC	CrR	CrRC	CrRP	CrRW
MDA (nmol/mg protein)	0.540?0.025[a]	0.848?0.044[b]	0.565?0.077[a]	0.606?0.048[a]	0.821?0.025[b]
Proteincarbonyls (nmol/mg protein)	2.32?0.153	2.09?0.117	2.17?0.071	2.11?0.094	2.40?0.102
GSH (?mol/mg protein)	3.58?0.157	4.11?0.212	3.39?0.458	3.24?0.186	4.03?0.096
GSSG (?mol/mg protein)	6.98?0.452	8.18?0.682	7.56?0.821	7.97?0.803	7.13?0.745
GSH/GSSG	0.518?0.026	0.526?0.057	0.379?0.047	0.434?0.061	0.598?0.066
CATALASE	0.110?0.006	0.102?0.008	0.107?0.008	0.114?0.006	0.099?0.006
SOD	7.79?0.428 [a]	6.12?0.553 [b]	6.21?0.249 [b]	7.30?0.553 [a]	6.94?0.299 [a]
GPx	0.228?0.014[a]	0.165?0.024[b]	0.184?0.016[a]	0.259?0.029[a]	0.215?0.017[a]

Values are mean SE (n=6)
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA

Table 4. Oxidative stress and antioxidant status in liver homogenate of different groups in female offspring

	CrC	CrR	CrRC	CrRP	CrRW
MDA (nmol/mg protein)	0.358? 0.053	0.347? 0.020	0.405? 0.033	0.340? 0.023	0.327? 0.024
Protein carbonyls (nmol/mg protein)	2.44? 0.143	2.53? 0.054	2.38? 0.135	2.65? 0.061	2.52? 0.103
GSH (?mol/mg protein)	3.74? 0.140	3.40? 0.268	3.60? 0.201	3.60? 0.361	3.17? 0.367
GSSG (?mol/mg protein)	6.93? 0.758	8.00? 0.660	6.64? 0.656	7.01? 0.413	8.63? 0.575
GSH/GSSG	0.589? 0.092^{a}	0.440? 0.050^{a}	0.562? 0.053^{a}	0.514? 0.044^{a}	0.376? 0.053^{b}
CATALASE	0.074? 0.004^{a}	0.049? 0.002^{b}	0.070? 0.003^{a}	0.066? 0.006^{a}	0.051? 0.002^{b}
SOD	7.79? 0.423^{a}	5.97? 0.393^{b}	6.18? 0.296^{b}	6.49? 0.366^{b}	6.30? 0.184^{b}
GPx	0.293? 0.018^{a}	0.207? 0.026^{b}	0.177? 0.012^{b}	0.179? 0.019^{b}	0.208? 0.067^{b}

Values are mean SE (n=6)
Means without a common superscript are significantly different at $p< 0.05$ by one way ANOVA

CONCLUSIONS

Chronic maternal Cr restriction significantly increased body fat %, specially the central adiposity (adiposity index) in the offspring in addition to altering adipocytokine levels in circulation and adipose tissue. In line with these findings, lipid metabolism was altered in these offspring as evident from increases seen in circulating triglyceride and FFA levels. However, maternal Cr restriction did not alter the expression of genes involved in adipogenesis / adiposity / lipid synthesis suggesting perhaps that the effect could be at the post-transcriptional / translational level.

In addition to increased body adiposity chronic maternal Cr restriction impaired glucose tolerance and increased insulin secretion (basal and glucose stimulated). That there were no changes at transcriptional level in the expression of genes regulating gluconeogenesis, glycogen synthesis and fatty acid synthase indicate that maternal Cr restriction induced effects may not be at transcription level. Indeed, significantly high plasma insulin levels were seen in CrR offspring and this appeared to be due to increased expression of genes Ins1 and Ins 2 in pancreas.

Maternal Cr restriction showed significant decrease in the 11 hsd1 gene expression in female CrR offspring (but not in males). Also, there was decrease in the antioxidant activity and increase in the MDA levels indicating that Cr restriction induced oxidative stress in these offspring. In general, the rehabilitation regimes corrected the insults partially.

8 HEALTH BENEFICIAL EFFECTS OF PLANT FOODS COMMONLY CONSUMED IN INDIA: NUTS AND OIL SEEDS

We are involved in generating a data base on the phenolic content and antioxidant activity (AOA) of plant foods commonly consumed in India. So far we have generated data on antioxidant activity of cereals, millets, legumes, pulses, roots, tubers and vegetables *(Annual Report 2005– 2008)*. The present report gives for the first time, data on total phenolic content and antioxidant activity (AOA) of nuts and oil seeds. Commonly consumed varieties of nuts and oil seeds were procured from three different shops from three different local markets of the twin cities of Hyderabad and Secunderabad.. The three samples from a given market were pooled and the pooled sample was considered as the sample of that market. Thus we have analysed three (pooled) samples of each food three different local markets and the data are presented as Mean SD, n = 3. Standard protocols described by us earlier *(Annual Report 2007-08)* were used for extraction and analysis of phenolic content and antioxidant activity parameters (FRAP and DPPH) reported in the present study. The results along with the botanical names of the foods analysed are given in Table 1 and the salient findings are as follows:

RESULTS

1. DPPH scavenging activity ranged from 0.20-286 mg/g of the food (trolox equivalent). Arecanut had the highest DPPH activity (286.22) followed by mustard seeds (11.55) and coconut water had the least activity (0.20 mg/ml) (trolox equivalent).

Table 1. Antioxidant activity of commonly consumed nuts and oil seeds
(Values are Mean ± SD, n=3)

Name of the nuts & oil seeds	Botanical name	Phenolic content mg/100g	DPPH (trolox equivalent mg/g)	FRAP (µmol/g)
Arecanut	*Areca catechnu*	10841.46? 2258.23	286.22? 46.49	151810.84? 27815.47
Coconut (dry)	*Cocos nucifera*	40.25?1.99	0.99?0.0	45.30?0.62
Coconut tender	*Cocos nucifera*	39.50?3.79	0.74?0.05	40.51?6.37
Coconut milk	*Cocos nucifera*	31.51?4.93	1.29?0.13	33.10?2.02
Coconut water	*Cocos necifera*	10.96?1.32	0.20?0.01	7.94?0.81
Gingelly seeds	*Sesamum indicum*	148.92?35.32	1.54?0.15	204.80?6.00
Linseed seeds	*Linum usitatissimum*	119.79?11.01	1.35?0.32	190.06? 40.53
Mustard seeds	*Brossicanigra*	725.28?38.44	11.55?0.82	995.20? 117.20
Niger seeds	*Guizota abyssinica*	143.95?3.06	1.54?0.03	192.36? 11.20
Safflower seeds	*Carthomus tinctorius*	599.97?51.53	2.28?0.33	466.73? 28.10
Sunflower seeds	*Helianthus annus*	207.95?7.33	8.50?1.26	293.21? 7.17
Water melon seeds	*Citrullus vulgaris*	74.60?11.00	0.54?0.04	58.32?5.59

Correlation	R	r^2%
TPC Vs DPPH	0.998	99.71
TPC Vs FRAP	0.997	99.53
DPPH Vs FRAP	0.999	99.85

2. FRAP acitivity also showed a wide range 7.94 -151810.84 µmol/g among the nuts and oil seeds analysed. The highest FRAP activity was in arecanut (151814.84 µmol/g) followed by 995.20 mol/g in mustard seeds, while the lowest FRAP activity was seen in coconut water (7.94 µmol/ml).
3. Phenolic content of nuts and oil seeds showed a wide range (10 -10841 mg/100g) among the 12 commonly consumed varieties of nuts and oilseeds studied. Interestingly, arecanut had the highest total phenolic content (10841.46) followed by mustard seeds (725.28). The lowest total phenolic content (TPC) was observed in coconut water 10.96 mg/100ml.
4. Significant correlation was observed between TPC and AOA with reference to DPPH and FRAP, the r values being 0.998-0.997 respectively.
5. The results indicate that total phenolic compounds may be important contributors to the AOA of nuts and oilseeds commonly consumed in India.

9 IMPORTANCE OF α-CRYSTALLIN HETEROPOLYMER IN THE EYE LENS: OLIGOMERIC SIZE, POLYDISPERSITY AND STABILITY

The small heat-shock protein α-crystallin isolated from the eye lens exists as a large (700 kDa) heteropolymer composed of two subunits, αA and αB, of 20 kDa each. Although trace amounts of αA-crystallin are found in other tissues, non-lenticular distribution of α-crystallin is dominated by the αB homopolymer. α-Crystallin is abundant in the eye lens of almost all vertebrates, reaching levels up to 50% of lens soluble proteins. Apart from its structural role, α-crystallin is known to have chaperone like activity. Impaired function of the lens due to partial or complete opacification is called **cataract**. Thus, the chaperone function is suggested to be instrumental in the prevention of cataract formation in the ocular lens. αA and αB subunits, form homo- and hetero-polymers according to the proportion of individual homopolymers mixed. Reconstituted as well as recombinant homopolymers of αA- and αB-crystallin are known to attain similar structural and functional integrity to that of native α-crystallin. However, it was reported that, despite high sequence homology, αA- and αB-crystallins behave differently with respect to their chaperone activity, hydrophobicity, structure and other physicochemical properties, particularly with increasing temperature. Earlier, we have demonstrated that though under normal conditions αA- and αB- homopolymers do not differ significantly with respect to their secondary structure and hydrophobicity, the αB-homopolymer has shown relatively higher chaperone-like activity (CLA). In contrast, the αA-homopolymer or the heteropolymer with a higher *α*A proportion (3:1 ratio) has shown greater CLA at elevated temperatures and also upon structural perturbation *(Biochem J,* ***414****, 453–460, 2008).* The main objective of the study is to understand the significance of 3:1 ratio of A / B crystallin in terms of its oligomeric size and thermal stability.

METHODOLOGY

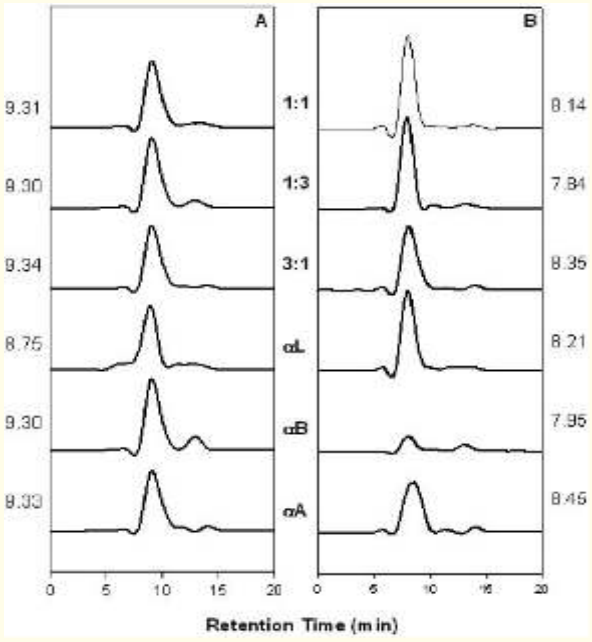
Recombinant αA- and αB-crystallins were expressed in *E. coli* and purified according to previously reported methods (*FEBS Lett. 2002, 522, 59-64*). Recombinant αA and αB-crystallins were mixed accordingly and incubated at 4°C for 60 min to obtain the heteropolymer with desired sub unit ratio. Goat αL-crystallin (native variant of 3:1 ratio)

was taken as a reference. Size exclusion chromatography of purified crystallins was performed on a TSKG4000 SWXL 7.8 x 300 mm column connected to a Shimadzu HPLC system. Light scattering of crystallin variants was monitored in a Perkin Elmer UV/Vis spectrophotometer at 85°C at 360 nm for 60 min. Differential scanning calorimetry (DSC) analysis of α-crystallin variants was carried out using a VP-DSC instrument scanning from 10-90°C at a scan rate of 1°C/min with 1 mg/ml protein. Dynamic light scattering (DLS) studies were performed on a 90° laser light scattering instrument (Viscotek-802) to determine the hydrodynamic radii (R_h).

RESULTS

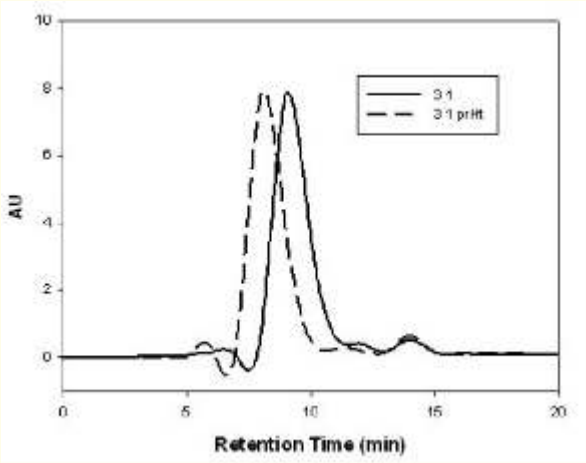
1. Though, oligomeric mass of native heteropolymer of α-crystallin is 800 kDa, homo and heteropolymers of recombinant α-crystallin seem to have an apparent molecular mass of 650 kDa which was not significantly different among the variants (Figure 1).

Fig 1. Size exclusion chromatography of α-crystallin variants on TSKG4000 column under normal conditions (*Panel A*) and up on preheating (*Panel B*)



2. Upon heating and cooling (preheating), oligomeric size of all combinations of α-crystallin has increased excepting B-crystallin (Figure 2). Upon preheating, αB-crystallin has precipitated where as the recombinant heteropolymer with 3:1 ratio of A and B remained in solution (Figure 2) indicating that A might impart stability to B-crystallin.

Fig 2. Size exclusion chromatography of normal and preheated A & B in 3:1 ratio on TSKG4000 column



3. Similarly at 85°C αB displayed highest light scattering compared to the other combinations. The light scattering was reduced with increasing proportions of αA in the reconstituted heteropolymer and it was least in the goat αL-crystallin (Figure 3).

Fig 3. Time course light scattering of α-crystallin variants at 85°C

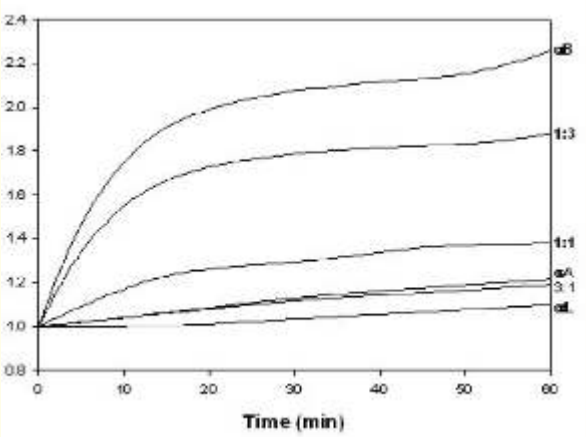
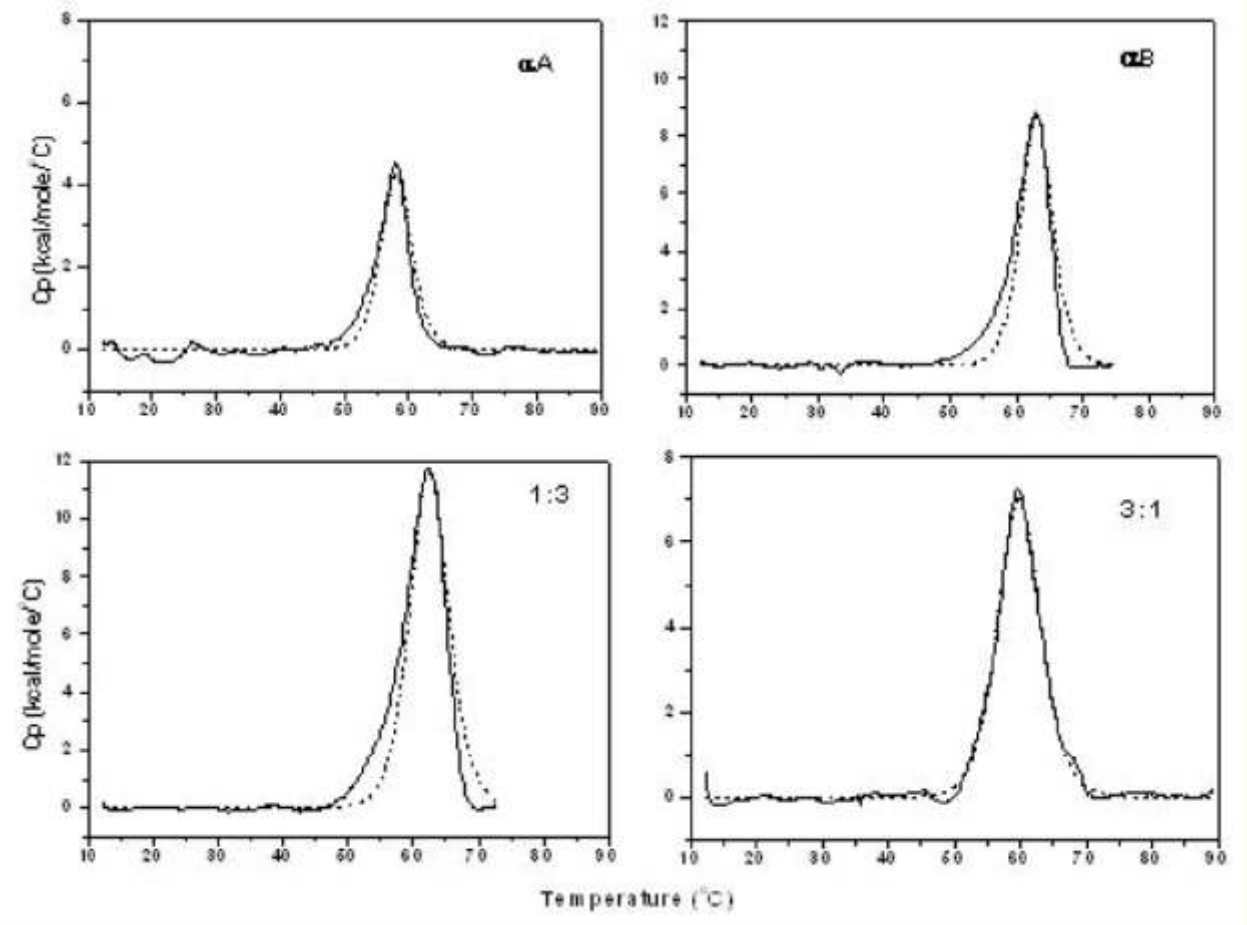


Fig 4. DSC thermograms of the α-crystallin variants



4. DSC data indicate that though, Tm of αB-homopolymer is higher compared to other combinations it precipitates at higher temperatures (Figure 4). However, the αA-homopolymer or the heteropolymer with a higher αA proportion (3:1 ratio) could withstand a rescan (data not shown). There was no significant difference the thermodynamic data obtained from DSC analysis between different variants of α-crystallin (data not shown).
5. Dynamic light scattering data have shown that αB and 1:3 got precipitated upon preheating whereas remaining variants showed either an increase or no change in the aggregate size. αL and 3:1 were comparable in size.

CONCLUSIONS

Based on our previous results and the present data the heteropolymer with higher *α*A proportion (3:1) or the αA-homopolymer seems to be better chaperones in protecting lens *β*- and *γ* -crystallins at both normal and elevated temperatures, which in turn is, supported by higher stability for these combinations. Thus lens might have favoured a combination of these qualities to achieve optimal protection under both native and stress (perturbed) conditions for which the heteropolymer with *α*A to αB in the 3:1 ratio appears to be better suited.

10 EXPRESSION OF A-CRYSTALLINS UNDER HYPERGLYCEMIC CONDITIONS: ROLE OF OXIDATIVE STRESS, TRANSCRIPTION FACTORS AND DIETARY ANTIOXIDANTS

α-Crystallin, a major lenticular protein, consists of two subunits αA and αB of each 20 kDa. αA- and αB-Crystallins belong to the small heat shock protein (sHSP) family and they share around 57% sequence homology. Interestingly, the presence of these proteins has also been demonstrated in a variety of non-lenticular and non-ocular tissues. αB-crystallin is shown to be present in several non-ocular tissues such as cardiac and skeletal muscle and, to a lesser extent, in brain, kidney, skin and lungs. In contrast to αB-crystallin, αA-crystallin is believed to be largely lens-specific. Although, the function of α-crystallin in non-lenticular tissues has not been demonstrated, it is believed that α-crystallin is associated with a variety of pathological conditions such as desmin-related cardiomyopathy, and most notably with the neuronal diseases. It has been reported that αB expression is found to be elevated under oxidative and other stress conditions. Oxidative stress induced expression of αB-crystallin as well as the presence of αB-crystallin in tissues with high oxidative potential (such as skeletal muscle, cardiac tissue and lung) suggests that the expression of αB-crystallin gene is related to oxidative stress. Diabetes is known to be associated with various metabolic stresses including oxidative stress. Previously, we have reported that expression of α-crystallins, particularly αB-crystallin, is elevated in several tissues of diabetic rat compared with non-diabetic rats (*Arch Biochem Biophys* **444**: 77-83, 2005). However, the stimulus for induced expression of α-crystallins in diabetic tissues it is not known. In general stress response is mediated by a family of transcription factors called heat shock factors (HSF). As a response to external stress stimuli, HSF monomers get trimerized and exported to nucleus and interact with heat shock element (HSE) there by activating transcription of the down stream heat shock gene(s). It should be noted that αB-crystallin promoter has canonical HSE and its expression could be controlled by HSF. Though, there are three known HSFs in mammals, HSF1, HSF2 and HSF4, it is not known that which particular HSF regulates αB-crystallin expression in hyperglycemia. In this report, we have investigated mechanism of elevated expression αB-crystallin, regulation by transcription factors and role of a dietary antioxidnat in eye lens and muscle of diabetic rats.

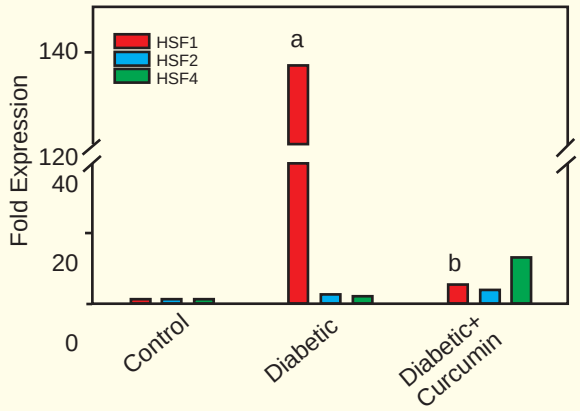
METHODOLOGY

Diabetes was induced in Wistar-NIN rats of 3-month-old by single intra peritoneal injection of STZ. Both control and diabetic rats (n=10) were fed on a semi synthetic AIN-93 diet where as another group of daibetic rats were fed with semi synthetic AIN-93 diet supplemented with 0.01% curcumin. All the three group of rats were maintained for 8 weeks and sacrificed to harvest eye balls and skeletal muscle. Total RNA was prepared from lens and muscle samples using Tri-reagent and reverse transcription was performed with total RNA and the product is used to perform real-time PCR using gene specific primers. Normalization and validation of the data were carried out using ß-actin as the housekeeping control. Each sample was analyzed in triplicate in individual assays performed on two or more occasions. Activities of skeletal muscle enzymes involved in antioxidant defense, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and protein carbonyl content were determined by standard methods.

RESULTS

1. Using real-time quantitative PCR we have assessed expression of HSF1, HSF2 and HSF4. Expression of these heat shock factors is substantially low in control tissues. Interestingly, while HSF1 expression is elevated many folds in diabetic muscle, HSF2 and HSF4 expression is not altered (Figure 1).

Fig 1. Expression of transcription factors HSF1, HSF2 and HSF4 in skeletal muscle by real-time PCR. The bars represent mean SD (n=4) and that do not share common super scripts differ significantly (*P* 0.01).



2. Similarly, HSF1 expression is also significantly elevated in diabetic lens (Figure 2).

3. The possible stimulus responsible for elevated expression of HSF1 in diabetic lens and muscle appears to be increased oxidative stress as there was a significant increase in protein carbonyl content and activities of SOD, GPx and GST in diabetic muscle (Table 1). In addition, earlier we have reported increased oxidative stress in the lens of diabetic rat.

4. Hence, elevated expression of HSF1 could be the factor that drives the up-regulation of αB-crystallin in dibaetic tissues.

5. Further, feeding of curcumin, a dietary antioxidant, substantiated the role of oxidative damage in inducing the expression of α-crystallin through HSF1, as curcumin not only attenuated the elevated expression of HSF (Figures 1 & 2) but also αB-crystallin (Figure 3) in diabetic eye lens and muscle.

Table 1. Protein carbonyl content and activities of antioxidant enzymes in skeletal muscle of control and diabetic rat. The data represent mean SD (n=4).

Parameter Diabetes	Control	
Protein carbonyls	9.2 ± 0.35	15.4 ± 0.36[a]
SOD	1.3 ± 0.08	1.8 ± 0.16 [a]
GPx	3.2 ± 0.25	4.4 ± 0.14 [a]
GST	2.6 ± 0.18	4.6 ± 0.04 [a]

Fig 2. Expression of transcription factor HSF1 in eye lens by real-time PCR. The bars represent mean SD (n=4) and that do not share common super scripts differ significantly (*P* 0.01).

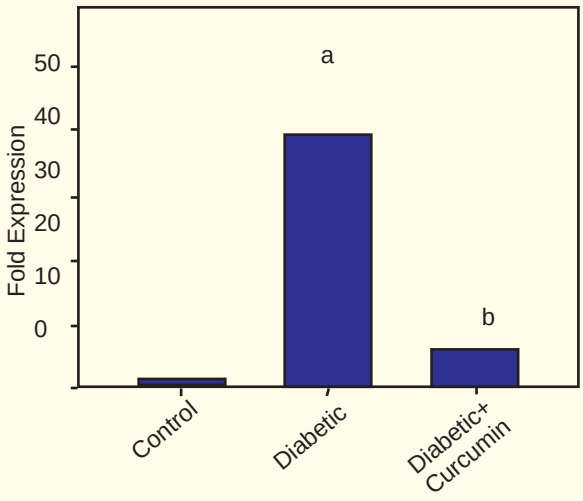
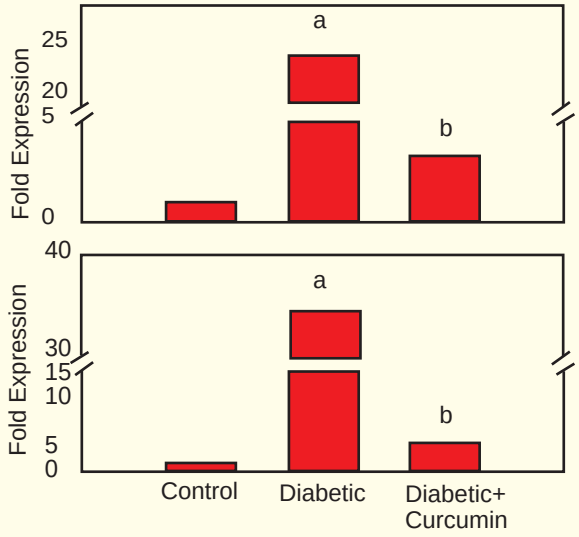


Fig 3. Effect of curcumin on expression of αB-crystallin in diabetic eye lens (top panel) and muscle (bottom panel). The bars represent mean SD (n=4) and that do

not share common super scripts differ significantly (*P* 0.01).



CONCLUSIONS

These results indicate that HSF1 might be the predominant transcription factor that is responsible for up regulation of αB-crystallin under hyperglycemic conditions.

Increased oxidative stress appears to be a major stimulus for the enhanced expression of αB-crystallin in the tissues of diabetic rats and elevated expression of α-crystallin may have a protective role against metabolic stress. Interestingly, feeding of curcumin, a dietary antioxidant, to diabetic rats attenuated the enhanced expression of αB-crystallin.

11 CHARACTERIZATION AND SIGNIFICANCE OF A NOVEL FATTY ACID ELONGASE, ELOVL4, OF THE EYE

A significant amount of the fatty acids are synthesized by the cytosolic enzyme complex fattyacid synthase (FAS) or taken up by the diet further elongated into very long chain fatty acids (VLCFA) in a four step reaction cycle by membrane bound enzymes predominantly located in the endoplasmic reticulum. Long chain polyunsaturated fatty acids (LC-PUFA) are involved in many biological functions including fetal growth and development, retinal function, brain development and probably lens transparency. Very long chain fatty acids (VLCFA) represent 30% of the total fatty acids in retina, brain and lens- indicating that they might play a crucial role in normal functioning of these specialized tissues. VLCFA biosynthesis is catalyzed by the enzyme system fatty acid elongases and the mammalian enzymes that elongate palmitic acid (16:0) and very long chain fatty acids (>C18) have been localized to the endoplasmic reticulum (ER). ELOVL4 (Elongation of Very Long Chain Fatty Acid 4) is a novel member of family of human fatty acid elongases, which is expressed only in tissues with high contents of very long chain fatty acids and whose functional role is currently not known. It has been reported that the ELOVL4 gene is highly conserved throughout evolution and is expressed in the photoreceptor cells of the retina in a number of species. Recently it was identified that a mutation (5-bp deletion) in *ELOVL4* gene can cause a particular form of macular degeneration. In our preliminary studies ELOVL4 expression was also observed in the lens tissue of the mouse. However it is not known whether ELOVL4 is present in the eye lens of other species. Further, the function and significance of Elovl4 in the eye lens or retina is not known. Thus, our main aim of the study was to understand the biological role of ELOVL4 in the eye.

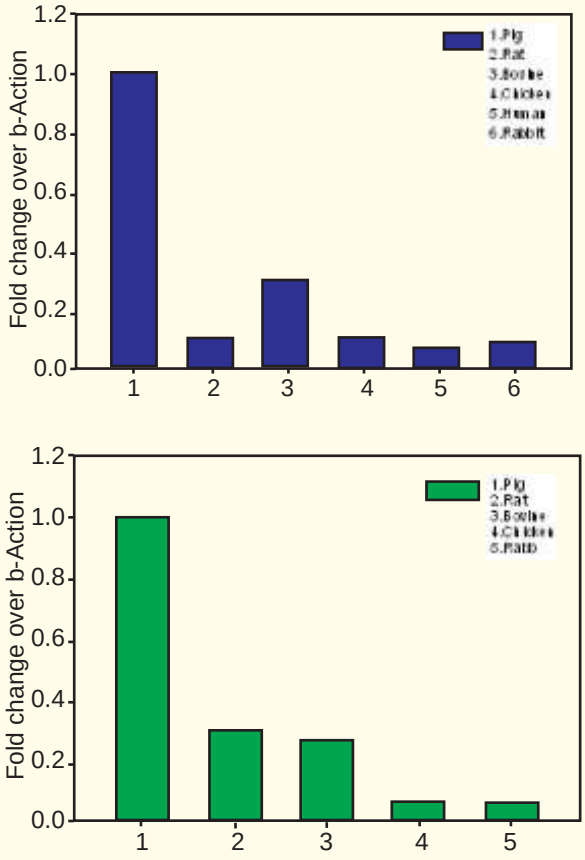
METHODOLOGY

Expression of ELOVL4 in eye lens and retina of different species was analyzed by quantitative real-time PCR using gene specific primers (forward primer- ATCTCCTGTTTGTGTGGCT and reverse primer- TCGTTTCCACCAAAGATATT). Fatty acid composition of lens and retina was analyzed by gas chromatography. In brief, total lipids were extracted from lens and retina by Bligh and Dyer method. Total lipids were subjected to alkaline methanolysis for subsequent analysis on GC. We predicted antigenic regions of ELOVL4 using bioinformatic tools and designed antigenic peptide sequences for raising antibodies against ELOVL4. These custom synthesized peptides were used for the production of polyclonal antibodies in rabbits. ELOVL4 gene from human brain cDNA library was PCR amplified with gene specific primers and cloned into pDEST15 vector using Invitrogen Gateway Technology. We have transformed GST-fusion construct of Elovl4 in pDEST15 vector into *E. coli* BL21 cells and protein expression was induced with 1mM IPTG for the expression of this protein. We have also attempted to express Elovl4 in Baculovirus expression system, as it is a mammalian membrane protein.

RESULTS

1. Based on real time PCR analysis, ELOVL4 gene expression was found to be high in pig lens and retina when compared to other species (Figure 1). Expression of this novel elongase is higher in retina compared to lens in the given species (Figure 1).
2. The fatty acid profile of lens and retina of pig and rat was analyzed and compared to find difference in fatty acid profile of these two species, as ELOVL4 expression was many folds higher in pig compared to rat. Further this may give indication of the substrate of ELOVL4.
3. Data on fatty acid composition by GC indicates that the total lipid content is relatively very high in lens and retina of rat when compared to pig tissues. Thus a direct comparison between rat and pig did not provide any specific information regarding the role of ELOVL4 in fatty acid analysis.

Fig 1. Expression of Elovl4 in eye lens (*top panel*) and retina (*bottom panel*) in different organisms



4. Nevertheless, comparison of fatty acid profile between lens and retina of given species (i.e, either rat or pig) has enabled us to cling on to the elongation reaction of Elovl4, as the expression of ELOVL4 was many folds higher in retina when compared to lens in the given species.
5. We found that the content of fatty acid with C30 carbon length was more in retina in

Table 1: Comparison of fatty acid profile between pig and rat eye lens and retina

	Pig Retina	Pig Lens	Rat Retina	Rat Lens
	?g/mg tissue			
16:0	0.97	0.52	2.78	0.786
16:1	0.04	0.028	0.042	0.06
18:0	0.83	0.13	3.89	0.35
18:1	0.79	0.50	1.58	1.35
18:2	0.32	0.348	0.47	0.103
20:0	0.04	-	-	0.022
20:3	0.022	-	0.0478	0.025
22:0	0.0007	-	-	0.062
20:4	0.48	0.055	0.769	0.078
24:0	0.002	-	0.096	0.086
24:1	-	0.02	0.275	0.048
22:5	0.048	0.036	0.006	0.062
22:6	1.14	-	4.09	0.037
Total	**4.74**	**1.92**	**15.61**	**3.54**
After 26:0	**0.0038**	**0.005**	**0.106**	**---**
After 28:0	**0.009**	**---**	**0.09**	**0.1**
After 30:0	**0.237**	**0.04**	**0.28**	**0.13**

both species when compared to lens. Thus, suggesting that ELOVL4 might be involved in the elongation of fatty acids with a chain length greater than C28.

6. Peptide polyclonal antibodies of ELOVL4 generated in rabbit were not good enough to use them for the characterization of ELOVL4.
7. We did not succeed in expressing Elovl4 in bacterial system.
8. We could express his-tagged Elovl4 in SF9 insect cell line using Baculovirus expression system (Figure 2). Studies are underway to purify the recombinant protein for subsequent characterization of

Fig.2. Expression of His-tagged in sf9 cells as probed by immunodetection

UI-Uninfected SF9 cells; CON-Sf9 cells infected with control virus;
E3- & E8-SF9 cells infected with pFASTBacHTB-ELOVL4

structural and functional aspects of ELOVL4.

CONCLUSION

Novel elongase, ELOVL4 might be involved in the elongation of fatty acids with a chain length greater than C28 in ocular tissues.

12 EFFICACY AND SAFETY EVALUATION OF DAG OIL: ROLE OF DAG OIL ON LIPID METABOLISM

Extensive research over the past 40 years has clearly established the role of dietary fat in health and disease conditions. Based on this various health agencies and professionals have made recommendations to cut down the over all fat intake and consume good quality fat with optimal levels of saturated, mono unsaturated and polyunsaturated fatty acids. Excessive intake of calories, especially through fat leads to obesity. Therefore, the current research focus is on understanding the link between the type of fat consumed and life style-related disease particularly obesity. Of late, the emphasis has been on the development of the edible oils with altered characteristics that influence the fat metabolism and affect body weight. Such products are diacylglycerol (DAG)-rich oils and medium-chain triacylglycerol (MCT)- rich oils. DAG-oil as the name indicates contains nearly 80% DAG and has pale yellow color and light bland flavor and tastes similar to conventional edible oils. The DAG contents of conventional oils vary significantly.

In view of these proven benefits due to DAG-oil consumption, it is deemed important to develop DAG-oil within India and evaluate its health benefits and safety aspects. In this context, IICT has successfully, adapted Japanese methodology for the development of DAG-oil. Further, due to availabilities of facilities and animal models of obesity at National Institute of Nutrition, this collaborative project has been proposed.

Hypothesis: DAG oil has hypolipidemic/ hypertriglyceridemia/ anti-obesity effects.

OBJECTIVES

1. To evaluate the efficacy of DAG-oil as an agent to protect against post-meal hypertriglyceridemia in normal Sprague-Dawley rats and mutant WNIN/Ob rats.
2. To evaluate the anti-obesity effect of DAG oil in WNIN/Ob rats

EXPERIMENTAL DESIGN

4months old male 16 Sprague-Dawley rats and 16 lean and 16 obese rats of WNIN/Ob strain were taken and divided into two groups consisting of 8 SD, 8 lean and 8 obese rats and they were given 10% TAG or DAG oil-containing diet.

Group A		
10% TAG oil diet		
SD	Lean	Obese
Group B		
10%DAG oil diet		
SD	Lean	Obese

Work done during the year

1. Impact of DAG oil on physical parameters

In obese rats, feeding of DAG oil-containing diet did not decrease the weight gain and adiposity index compared to TAG oil-containing diet.

Further, daily food consumption was not altered by consumption of either DAG or TAG-oil containing diet (Table 1).

2. Impact of DAG oil on biochemical parameters

DAG-oil diet administration had no impact on plasma lipid profile in any of the rats strain used, when compared to TAG-oil diet fed rats (Table 2).

Table 1. Impact of DAG oil on physical parameters

Physical parameters	TAG-diet			DAG-diet		
	SD	Lean	Obese	SD	Lean	Obese
Initial body weight (g)	346.5±64.9	196.7±36.5	394.6±41.7	348.2±56.6	201.3±36.5	395.3±39.3
Final body weight (g)	438.6±44.0	446.2±31.5	761.1±61.3	457.2±43.8	408.8±35.5	774.7±47.8
Weight gain (g)	92.1±86.9	249.5±37.5	366.5±59.8	109.0±58.3	207.6±45.9	379.4±55.8
Adiposity index (%)	6.8±2.0	10.4±3.0	60.9±15.4	8.8±1.7	8.8±2.9	64.5±16.4

Values are means ± SD of 8 rats from each group. One way ANOVA was performed using SPSS software (Version 10.0). Groups compared: TAG-diet Vs DAG-diet with respect to their strain and phenotypes

Table 2. Impact of DAG oil on biochemical parameters

Plasma lipid profile (mg/dL)	TAG-diet			DAG-diet		
	SD	Lean	Obese	SD	Lean	Obese
Cholesterol	89.0±6.9	73.4±13.5	98.3±16.5	82.1±12.3	71.3±12.0	92.0±17.6
HDL-C	70.1±12.0	47.6±8.7	77.1±15.2	71.6±16.2	47.8±14.9	71.8±14.4
Triglycerides	58.6±18.1	76.1±39.1	214.3±53.7	60.5.0±13.2	80.8±41.1	233.7±77.7

Values are means ± SD of 8 rats from each group. One way ANOVA was performed using SPSS software (Version 10.0). Groups compared: TAG-diet Vs DAG-diet with respect to their strain and phenotypes.

CONCLUSION

In conclusion, results of the present study suggest that DAG oil used in this experiment does not possess hypotrigly-ceridemic and anti-obesity effects.

13 ROLE OF *SCAVENGER RECEPTOR CLASS B TYPE 1 (SR-B1)* IN REVERSE CHOLESTEROL TRANSPORT AND OTHER PHYSIOLOGICAL FUNCTIONS IN WNIN/Ob RAT MODEL: IMPACT OF VITAMIN A

Obesity is a multi-factorial disorder, resulting out of imbalanced energy homeostasis that leads to accumulation of excess energy as fat. In obesity unfavorable interactions between genetic and environmental factors impact food intake regulation, energy regulation and adipogenesis.

Nutrient interventions are effective in combating obesity and its associated disorders without causing any side effects. Previous studies from NIN clearly showed that feeding of high but non-toxic (129 mg vitamin A/kg diet) levels of vitamin A in the diet could effectively reduce body weight gain, adiposity index and plasma high density lipoprotein cholesterol (HDL-C) as well as total cholesterol (TC) levels in obese rats of WNIN/Ob strain (an animal model of obesity). So, in the present study, the impact of various doses of vitamin A on thermogenesis, obesity and HDL cholesterol metabolism were examined.

AIMS & OBJECTIVES

- Effect of various doses of vitamin A on thermogenesis and brown adipose tissue (BAT mitochondria and UCP1) in male WNIN/Ob rats.
- Effect of various doses of vitamin A on Reverse cholesterol transport (RCT) pathway in male lean and obese rats of WNIN/Ob strain.
- Effect of various doses of vitamin A on adiposity and obesity associated genes (SCDI, SREBP, MTP, LPL, FAS) in male WNIN/Ob rats.
- Effect of various doses of vitamin A on adipose tissue apoptosis in male WNIN/Ob rats.

STUDY DESIGN

Five-month-old male 32 lean and 32 obese rats of WNIN/Ob strain were obtained from National Centre for Laboratory Animal Sciences (NCLAS) and broadly divided into two groups A and B. Each group was further subdivided into four groups (I, II, III and IV), consisting of 8 animals each. Group I received 2.6 mg and served as the diet for control rats, group II received 26 mg, group III received 52 mg and group IV received 129 mg of vitamin A/kg diet; as retinyl palmitate respectively. Animals were fed on their respective diets for a period of four and half months. Food and water were provided *ad libitum*. Daily food intake and weekly body weights were recorded. At the end of four and half months, rats were killed by cervical dislocation after 12 h fasting. Blood was collected from the supra-orbital sinus via the inner canthus by fine heparinised capillary tube and allowed to stand and then centrifuged to separate plasma. Liver, adrenal gland and various adipose tissues were excised, weighed, rapidly frozen in liquid nitrogen and stored at -80°C until analysis.

GROUP A (LEAN)			
AI (n=8)	AII (n=8)	AIII (n=8)	AIV (n=8)
2.6 mg vitamin A/kg diet	26 mg vitamin A/kg diet	52 mg vitamin A/kg diet	129 mg vitamin A/kg diet
GROUP B (OBESE)			
BI (n=8)	BII (n=8)	BIII (n=8)	BIV (n=8)
2.6 mg vitamin A/kg diet	26 mg vitamin A/kg diet	52 mg vitamin A/kg diet	129 mg vitamin A/kg diet

METHODOLOGY

⋆ *Detection of DNA fragmentation in white adipose tissue*

WAT (200 mg) was homogenized in 1ml of lysis buffer (10mM Tris-HCl, 10mM EDTA, and 0.5% triton X-100, pH 8.0) in a glass-Teflon homogenizer and incubated on ice for 20 min. After centrifugation at 14000g for 15 min at 4^0C, the fat cake was removed and the supernatant containing fragmented (soluble) DNA was transferred to a new tube. The pellet was further processed to isolate genomic DNA using DNAzol (Molecular Research Center,USA). The fragmented and the genomic DNA fractions were loaded onto a 2% agarose gel and run at 128V (8 V/cm). After electrophoresis, the gel was stained with ethidium bromide and visualized with UV light using the DNR MiniBis Pro imager.

⋆ *Western blot of Bcl-2 and Bax*

WAT was homogenized with lysis buffer (50 mM Tris (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 1% SDS and 5% protease inhibitor cocktail (Sigma Chemical Co.), and centrifuged at 800 g for 10 min. Protein (50 g) from adipose lysate was separated on SDS-12% polyacrylamide gel then transferred onto nitrocellulose membranes. Equal loading of protein and transfer were ensured by staining membranes with Ponceau S (Sigma). Bax and Bcl2 levels were detected using specific polyclonal antibodies. Blocking and development of the immunoblots were performed using an enhanced chemilumine-scence western-blotting analysis system (Amersham Biosciences), with horseradish peroxidase-conjugated secondary antibodies (Sigma). Bands were visualized by chemilumine-scence. The intensity of developed bands was quantified by use of GS-710 Imaging Densitometer; Bio-Rad, Hercules, CA, USA).

⋆ **Immunoblotting of nuclear transcription factors**

100 mg of liver and 50 mg of BAT tissue were homogenized in lysis buffer [20mM Tris-HCl; pH 7.5, 2mM $MgCl_2$, 250mM sucrose and 5% protease inhibitor cocktail (Sigma-Aldrich, St.Louis, MO, USA)] and centrifuged at 700*g* for 10 minutes at 4^0C to obtain nuclear fraction. The pellet obtained was further subjected to extraction. A constant amount of membrane protein (80-100g) was separated on 10% SDS-PAGE, and transferred to nitrocellulose membrane (GE healthcare UK limited, Buckinghamshire, UK). The membranes were blocked and incubated with anti-RARα, anti-RXRα, anti-PPARα and anti-LXRα polyclonal antibodies (Santa Cruz, California, USA). The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hr. The bands were visualized by chemiluminescence method (ECL advance western blotting detection kit, GE healthcare UK limited, Buckinghamshire, UK).

⋆ *Western blotting of SR-BI and ABCA1*

Nearly 100mg of liver was homogenized in 10 vol. buffer [20mM Tris-HCl (pH 7.5), 2mM $MgCl_2$, 0.25 M sucrose, and 5% (v/v) protease inhibitor cocktail] and centrifuged (800 X *g*) for 10 min, and the supernatant was centrifuged for 60 min at 1,00,000 X *g*. The resulting pellet was washed with buffer to remove lipids, and dissolved in 0.1M phosphate buffer (pH 7.5) and the membrane fraction was used for immuno-blotting of SR-BI and ABCA1. A constant amount of membrane protein (100g) was solubilized in Laemmli buffer, separated on 10% SDS-PAGE and transferred to nitrocellu-lose membrane. Immunoblot analysis was done by using anti-SR-BI and anti-ABCA1 polyclonal antibodies as displayed above.

Glycerol 3-phosphate dehydrogenase activity in liver and RP-WAT

Briefly, tissues were homogenized in Tris-Cl buffer (pH 7.2) containing 1mM EDTA and 1mM 2-mercaptoethanol with protease inhibitor cocktail (Sigma) added at 5% level. Homogenate was centrifuged at 800g for 12min at 4^0C and the supernatant was used for

the determination of GPDH activity. Concentration of protein in the samples was estimated by Bradford method. Enzyme activity was measured as per the method by White et al and expressed as nanomoles of NADH oxidized per minute per milligram protein (nmoles/min/mg protein).

★ ***Enzyme activities***

Activities of LCAT (plasma), HL (liver), LPL (RPWAT) and MTP (liver) were analyzed as per manufacturer's protocol using kits purchased from Roar biomedical Inc.

★ ***Statistical analysis***

Results are expressed as means ± S.E. Statistical significance was determined by one-way ANOVA and F ratios were considered significant at the *P* ?0.05 level.

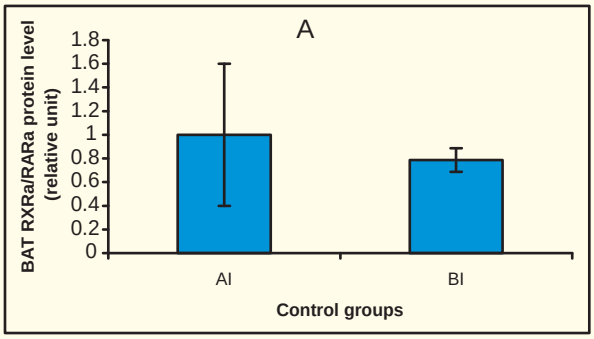
RESULTS

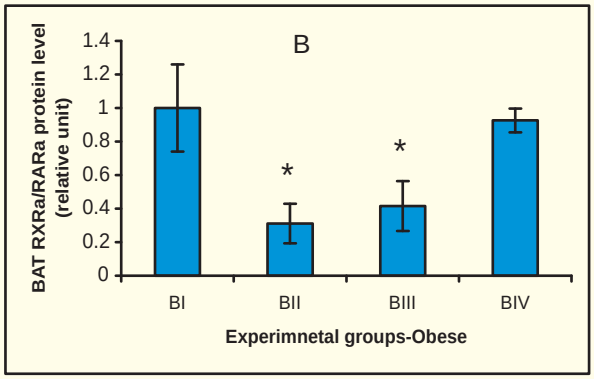
Effect of vitamin A on BAT nuclear factors RXR and RAR levels

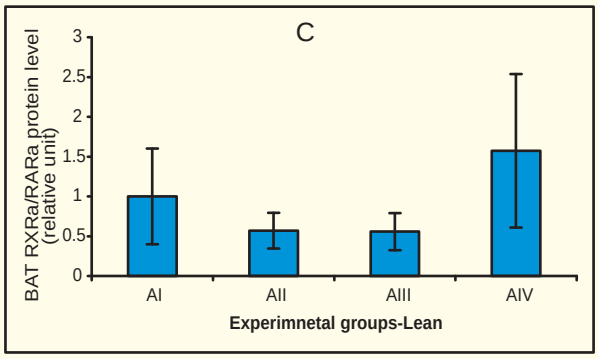
Obese control rats (BI) showed an under-expression of nuclear receptors RARα and RXRα compared to lean control rats (AI). Feeding of obese rats with various doses of vitamin A led to an over-expression of RARα, while no statistically significant changes were seen in vitamin A-treated lean rats. Group BII and BIII showed an under-expression of RXRα, while BIV showed an over-expression of RXRα upon vitamin A feeding compared to BI (control obese rats). Vitamin A treatment in lean rats did not result in any changes in RXRα protein levels.

The RXRα/ RARα ratio was calculated because high ratios indicate that RXRa is promoted over RARa expression, thus favouring adipogenesis, whereas low ratios means the opposite, indicating that adipogenesis is inhibited. There were no significant differences in the RXRα/ RARα ratio of lean and obese control rats. Vitamin A treatment to obese rats (groups BII and BIII) markedly reduced the RXRα/ RARα ratio, while no changes were seen in BIV compared to their respective controls (BI). Similarly, no changes were seen in RXRα/ RARα ratio upon vitamin A treatment in lean rats (Fig. 1).

Figure 1. Effect of vitamin A on BAT nuclear factors RXR and RAR levels







BAT RXR/RAR ratio quantified densitometric values are expressed relative to a value of 1.
A: Lean control vs obese control
B: Obese control vs treated doses
C: Lean control vs treated doses

Values are means ± S.E. for three to four rats.
* significant at *P* ?0.05 level (by one way ANOVA)

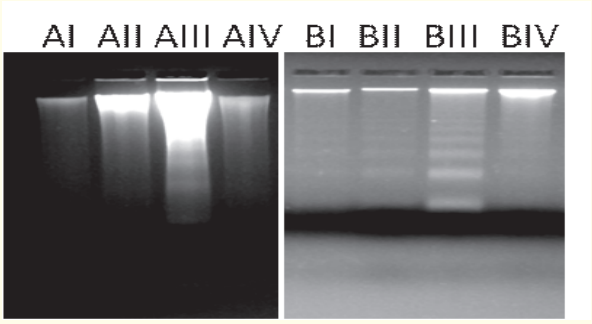
Effect of vitamin A challenging on DNA fragmentation in white adipose tissue

DNA fragmentation was not observed in the RPWAT, EPWAT and SCWAT of lean (AI) and obese (BI) rats, which received the control diet (2.6mg/kg diet). Similarly, lean rats supplemented with various doses of vitamin A did not exhibit any DNA fragmentation in any of the WAT depots except RPWAT of lean (AIII) rats receiving 52 mg of vitamin A/kg diet (**Figure**).

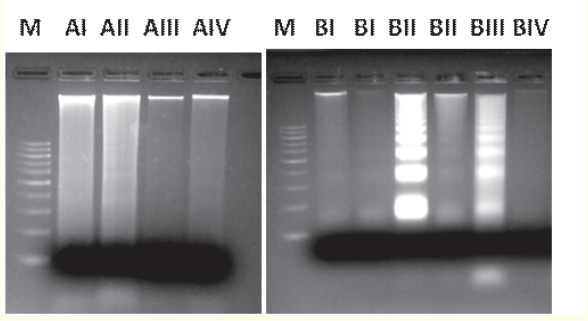
Obese rats maintained on various doses of vitamin A (BII, BIII and BIV) exhibited nucleosomal DNA fragmentation in SCWAT (**Figure**), while obese rats maintained on 26 mg (BII) and 52 mg of vitamin A/kg diet (BIII) showed marked DNA fragmentation in EPWAT (**Figure**). On the contrary, no marked nucleosomal DNA fragmentation was observed in the RPWAT of obese rats challenged with vitamin A.

Figure 3.11: Effect of vitamin A on DNA fragmentation in WAT

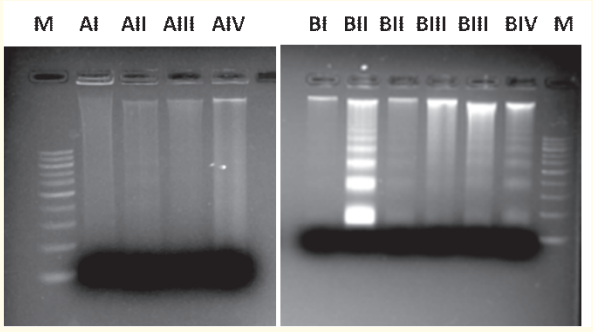
(A) RPWAT



(B) EPWAT



© SCWAT



Representative picture of inter-nucleosomal DNA fragmentation in (A) RPWAT, (B) EPWAT and (C) SCWAT (n=3).

Effect of vitamin A supplementation on apoptosis-related proteins in white adipose tissue

In lean (AI) and obese rats (BI) receiving normal dose of vitamin A through diet, the expression levels of Bcl2 were comparable, while there was a significant increase in Bax expression levels in RPWAT and SCWAT of obese rats (BI) receiving 2.6 mg vitamin A/kg diet compared to lean counterparts (AI) maintained on identical diet. In EPWAT, western blot analysis of apoptosis-related proteins revealed comparable expression levels of Bcl2 and Bax proteins in control diet fed lean (AI) and obese (BI) rats. Thus, the control obese rats showed a significantly decreased Bcl2 to Bax ratio compared to lean controls in both RPWAT and SCWAT (**Figure 3.13A**).

Vitamin A supplementation had no effect on RPWAT Bcl2 and Bax expression levels of lean and obese rats. As a result, the ratio of Bcl2 to Bax in this white adipose tissue depot was not altered upon vitamin A feeding in lean and obese rats as compared to their respective controls (AI and BI respectively) (**Figure 3.13B and 3.13C**).

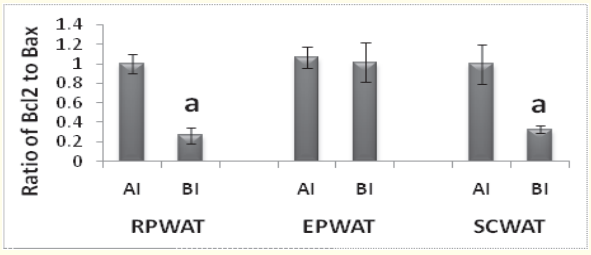
No changes were seen in EPWAT Bcl2 and Bax expression levels of vitamin A supplemented lean rats. However, obese rats (BIII) challenged with 52 mg/kg diet alone had significantly decreased Bcl2 levels and

increased Bax expression levels, as compared to control obese rats receiving 2.6 mg/kg diet (BI). Thus, the Bcl2 to Bax ratio was significantly reduced in EPWAT of obese rats (BIII) receiving 52mg of vitamin A/kg diet as compared to that of control obese rats (BI) (**Figure 3.13C**).

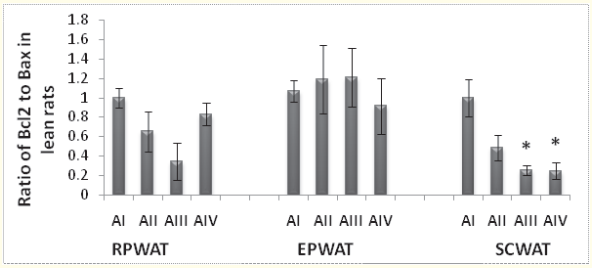
In SCWAT, the Bcl2 and Bax expression levels in obese rats (BIII) supplemented with 52mg of vitamin A/kg diet decreased and increased respectively compared to control diet fed obese rats (BI). No changes in Bcl2 and Bax expression levels were observed in obese rats supplemented with 26mg and 129mg of vitamin A/kg diet. Lean rats supplemented with 52mg and 129mg of vitamin A/kg diet had significantly reduced Bcl2 and increased Bax expression levels, while no changes were observed in lean rats receiving 26mg of vitamin A/kg diet when compared to control diet-fed lean rats (AI). Thus, the ratio of Bcl2 to Bax in SCWAT was significantly lower in obese rats maintained on 52 mg (**Figure 3.13C**) and in lean rats supplemented with 52 mg (AIII) and 129 mg (AIV) (**Figure 3.13B**) of vitamin A/kg diet compared to their respective control rats (i.e., BI and AI).

Figure 3.13: Bcl2-Bax ratio in WAT

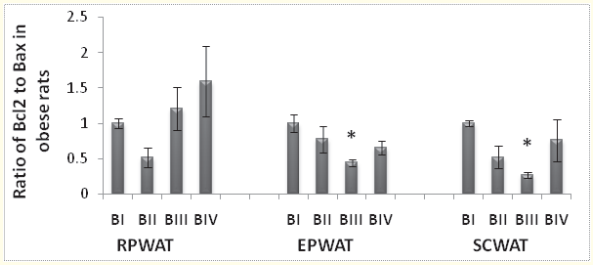
(A)



(B)



(C)



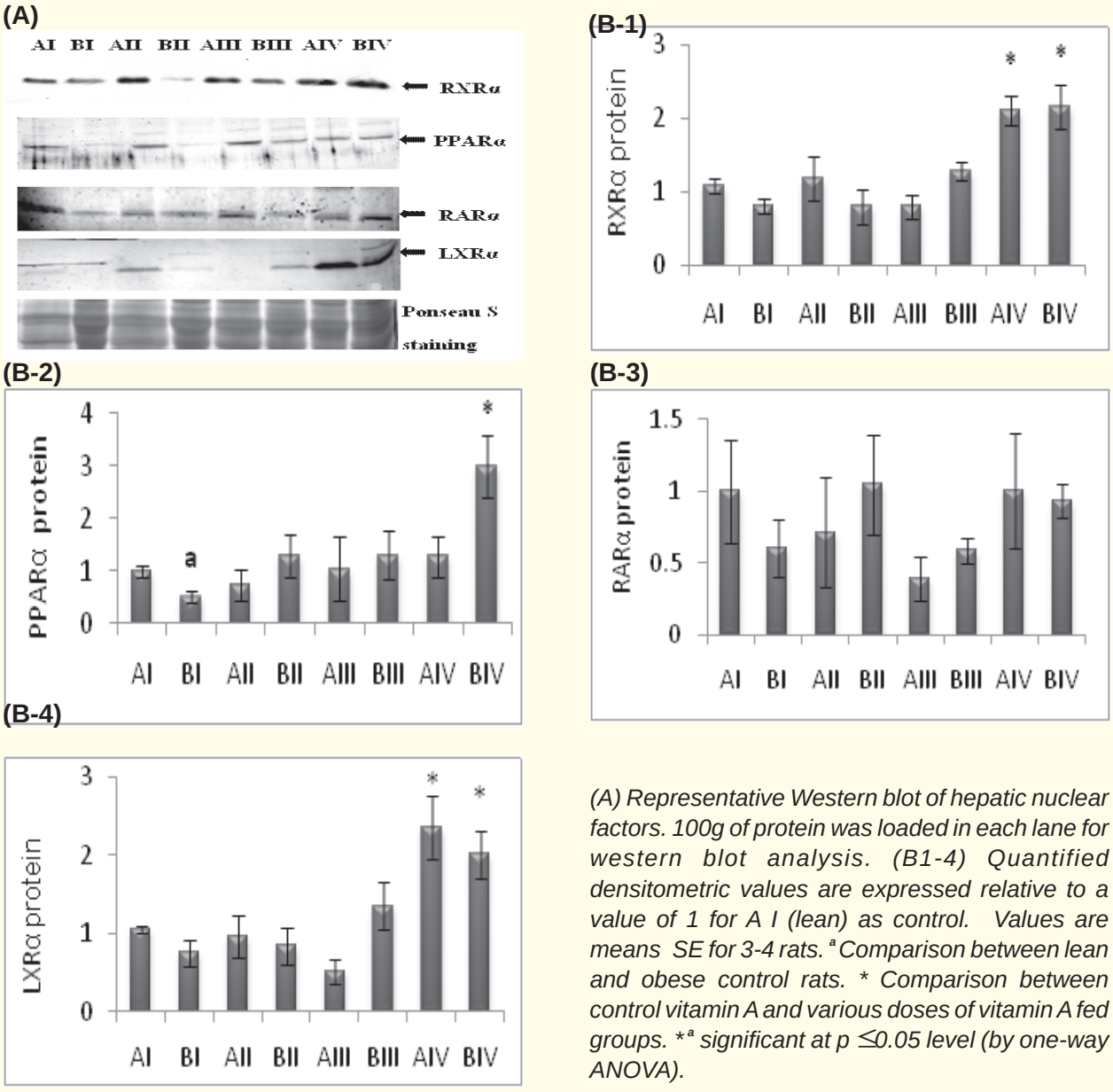
(A) Bcl2-Bax ratio between lean (AI) and obese (BI) control rats in various WAT depots, (B) Bcl2-Bax ratio between vitamin A treated lean rats in various WAT depots, (C) Bcl2-Bax ratio between vitamin A treated obese rats in various WAT depots. Quantified densitometric values are expressed relative to a value of 1. Values are means ± S.E. for three to four rats. [a] *Significant at $p \leq 0.05$ level. [a] Comparison between lean and obese control rats (Student's *t* test). * Comparison between control vitamin A and various doses of vitamin A fed groups.

Regulation of hepatic nuclear factors by vitamin A

Hepatic expression levels of PPARα, involved in peroxisomal oxidation of fatty acids, were down-regulated in obese rats (BI) as compared to age and sex-matched lean rats (AI). Hepatic RXRα and RARα levels tended to be lower in obese rats (BI) compared to lean phenotype (AI), while LXRα levels in the liver were similar in lean and obese phenotypes (AI and BI respectively) (**Figure**).

Interestingly, feeding of obese rats (BIV) with the highest dose of vitamin A (i.e. 129 mg/kg diet) resulted in increased hepatic PPARα, LXRα and RXRα levels (**Figure**). Further, lean rats treated with identical dietary regimen resulted in augmented hepatic LXRα and RXRα levels as compared to control diet-fed lean rats, while PPARα expression levels remained unaltered. No changes in the expression levels of hepatic PPARα, LXRα and RXRα were observed in lean and obese rats treated with 26 and 52 mg of vitamin A (**Figure**).

Figure: Impact of dietary vitamin A on hepatic nuclear transcription factors.

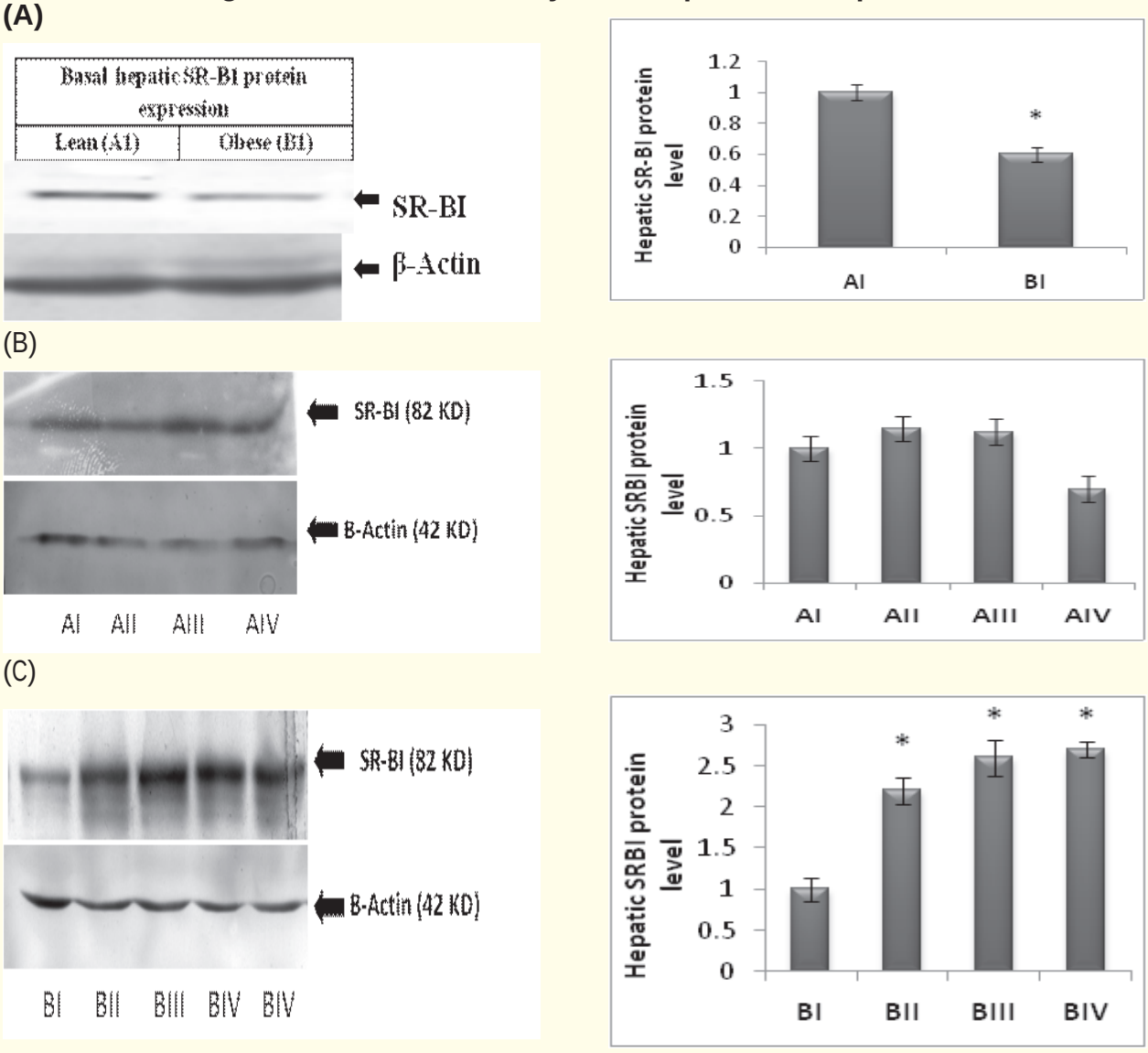


*(A) Representative Western blot of hepatic nuclear factors. 100g of protein was loaded in each lane for western blot analysis. (B1-4) Quantified densitometric values are expressed relative to a value of 1 for A I (lean) as control. Values are means SE for 3-4 rats. [a] Comparison between lean and obese control rats. * Comparison between control vitamin A and various doses of vitamin A fed groups. *[a] significant at p ≤0.05 level (by one-way ANOVA).*

Impact of Dietary Vitamin A on hepatic SR-BI receptor expression

To investigate the effect of various doses of vitamin A on hepatic SR-BI receptor expression, immunoblot analysis was done. We found an under-expression of hepatic SR-BI protein level in obese rats (BI) as compared to their lean counterparts (AI) (**Figure**). However, feeding of various doses of vitamin A resulted in over-expression of hepatic SR-B1 in obese phenotype (BII, BIII and BIV) as against their respective obese control rats (BI) receiving a diet with 2.6 mg of vitamin A/kg diet (**Figure**). However, in lean rats hepatic SR-BI expression levels were comparable in all the groups with no marked impact by vitamin A feeding (**Figure**).

Figure: Western Blot analysis of hepatic SR-B1 protein



(A) Representative western blot of basal hepatic SR-B1 protein. Quantified densitometric values are expressed relative to a value of 1 for A I (lean) as control. Representative western blot of hepatic SR-B1 protein in vitamin A supplemented lean (B) and obese (C) rats. Values are means SE for 3-4 rats. [a] *Significant at P 0.05 level Groups compared Lean Vs Obese. * Significant at P 0.05 level Groups compared normal vitamin A Vs various doses of vitamin A.*

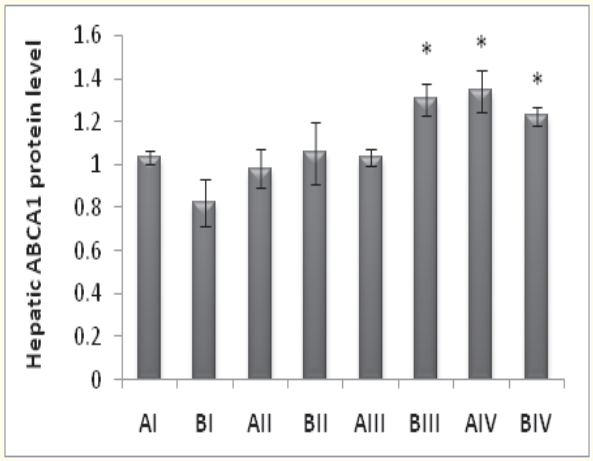
Impact of dietary vitamin A on hepatic ABCA1 expression

Hepatic ABCA1 protein levels were found to be slightly lower in obese rats (BI) (statistically not significant) compared to control diet-fed lean rats (AI). Chronic vitamin A supplementation with various doses of vitamin A resulted in over-expression of hepatic ABCA1 in obese rats receiving 52mg and 129mg of vitamin A/kg diet (BIII and BIV respectively) and in lean rats receiving 129mg of vitamin A/kg diet (AIV) when compared with their respective controls (AI and BI) (**Figure**).

Effect of vitamin A supplementation on hepatic ABCA1 protein

(A)

(B)



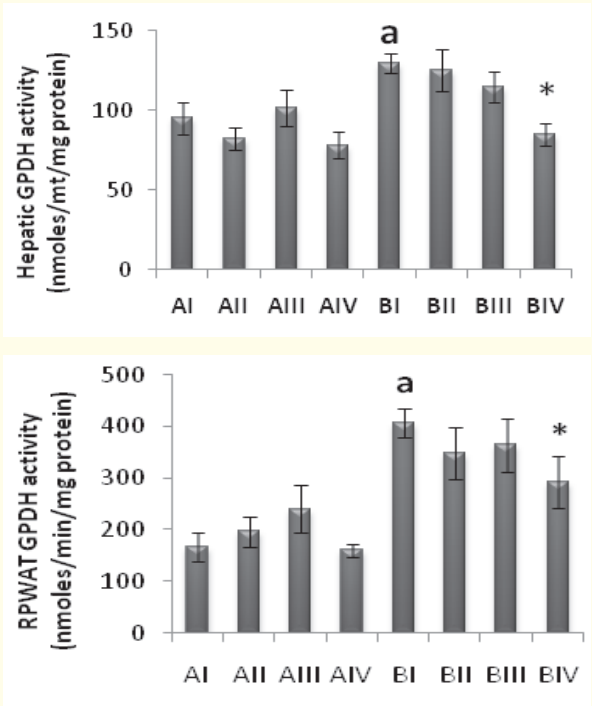
*(A) Representative Western blot of hepatic ABCA1 protein. 100g of protein was loaded in each lane for western blot analysis. (B) Quantified densitometric values are expressed relative to a value of 1 for A I (lean) as control. Values are means SE for 3 rats. [a] * Significant at P 0.05 level. [a] Comparison between lean and obese control. * Comparison between control vitamin A and various doses of vitamin A fed groups.*

Regulatory role of dietary vitamin A on GPDH activity

RPWAT-GPDH activity was 2.5 fold higher in obese rats (BI) compared to their lean littermates (AI) (**Figure**). Vitamin A feeding to lean rats did not result in any change in the GPDH activity compared to lean control rats. Interestingly, vitamin A at a dose of 129 mg resulted in 30% decrease in GPDH activity in obese rats (BIV), while no marked changes were observed in obese rats receiving 26 (BII) and 52 mg (BIII) compared to control diet-fed obese rats (BI) (**Figure**).

The endogenous TG synthesis in liver was studied by measuring hepatic GPDH activity. The activity of the enzyme was 35% higher in the liver of obese rats (BI) as compared to their lean littermates (AI) (**Figure**). Vitamin A feeding to lean rats did not result in any change in the hepatic GPDH activity as against control lean rats receiving 2.6 mg of vitamin A/kg diet. Interestingly, vitamin A feeding to obese animals at a dose of 129mg (BIV) resulted in 30% decrease in hepatic GPDH activity, while no change was observed in obese rats receiving 26 (BII) and 52 mg (BIII) as compared to control diet-fed obese rats (BI) (**Figure**).

Effect of dietary vitamin A on GPDH activity in liver and RPWAT

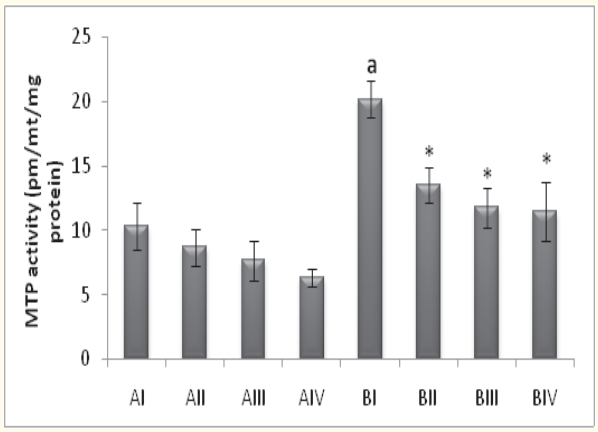


*Data represents the means ± SD of 6-8 rats from each group. [a] Comparison between lean and obese control rats. * Comparison between control vitamin A and various doses of vitamin A fed groups. [a] * significant at p ≤0.05 level (by one-way ANOVA). (A) hepatic GPDH activity, (B) RPWAT-GPDH activity*

Regulatory role of dietary vitamin A on MTP activity

Accumulation of TG and cholesterol in the liver of obese rats (BIV) upon vitamin A supplementation prompted us to look at the level of hepatic MTP activity in these rats. We observed that vitamin A can impact lipid metabolism by reducing the activity of MTP. Feeding of obese rats with various doses of vitamin A (BII, BII and BIV) resulted in 33%, 42% and 43% decreased hepatic MTP activity compared to control diet-fed obese rats (BI) (**Figure**). As a result, vitamin A supplementation decreased the levels of hepatic MTP activity in obese rats which were comparable to lean rats. On the other hand, there was only a trend towards decreased MTP activity in lean rats receiving various doses of vitamin A as against lean control rats (**Figure**).

Figure: Effect of dietary vitamin A on hepatic MTP activity in liver

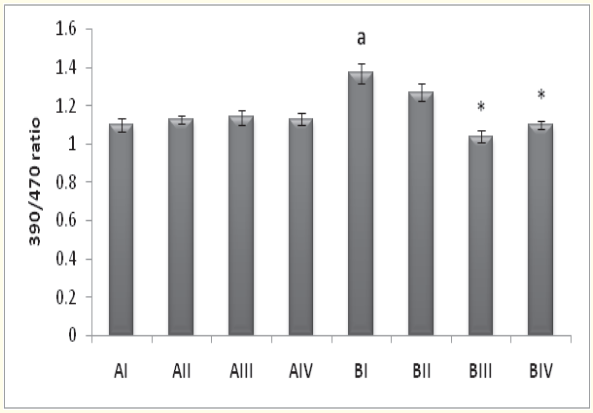


*Data represent the means ± SE of 6-8 rats from each group. [a] Comparison between lean and obese control rats. * Comparison between control vitamin A and various doses of vitamin A fed groups. [a] * significant at p ≤0.05 level (by one-way ANOVA).*

Impact of dietary vitamin A on plasma* LCAT *activity

There was a 25% increase in the plasma LCAT activity of obese rats (BI) compared to the control lean rats (AI), when measured using an exogenous reconstituted HDL substrate. Furthermore, vitamin A supplementation resulted in significant decrease in the LCAT activity of obese rats receiving 52 and 129 mg of vitamin A/kg diet compared to their respective controls (BI). Although, a decrease in the LCAT activity was also observed in obese rats supplemented with 26mg of vitamin A/Kg diet, the decrease was not statistically significant (**Figure**). No effect was seen in the LCAT activity in lean rats upon vitamin A supplementation (AII, AIII and AIV) as compared to their lean counterparts maintained on control diet with 2.6 mg/kg diet (AI) (**Figure**).

Figure: Effect of vitamin A supplementation on plasma LCAT activity

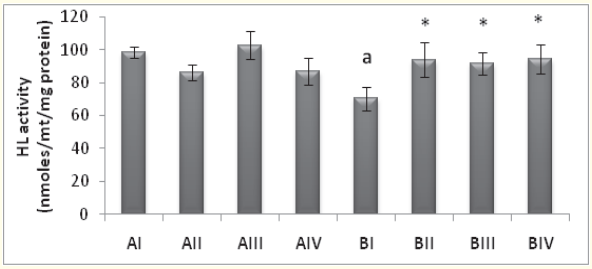


*Data represent the means ± SE of 5 rats from each group. (A) Comparisons were made between phenotypes, between control and treated groups. [a] * Significant at P 0.05 level. [a] Comparison between lean and obese control. * Comparison between control vitamin A and various doses of vitamin A fed groups.*

Impact of dietary vitamin A on hepatic lipase activity

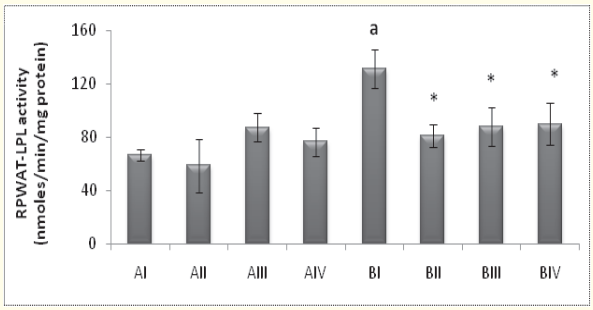
Obese rats (BI) had lower hepatic lipase activity as compared to their lean counterparts (AI). Vitamin A supplementation resulted in increased hepatic lipase activity in obese rats from BII, BIII and BIV groups as compared to obese control rats (BI) (**Figure**). No change was observed in hepatic lipase activity of lean rats (AII, AIII and AIV) treated with various doses of vitamin A as compared to their respective age and sex-matched lean controls receiving 2.6 mg/kg diet (AI) (**Figure**).

Figure :Effect of vitamin A on Hepatic lipase (HL) activity



*Data represents the means ± SD of 7 rats from each group. [a] * Significant at P 0.05 level. [a] Comparison between lean and obese control. * Comparison between control vitamin A and various doses of vitamin A fed groups.*

Figure: Effect of dietary vitamin A on RPWAT-LPL activity



*Data represents the means ± SD of 6-8 rats from each group. [a] Comparison between lean and obese control rats. * Comparison between control vitamin A and various doses of vitamin A fed groups. [a] * significant at p ≤0.05 level (by one-way ANOVA).*

Regulatory role of dietary vitamin A on LPL activity

Obese rats of WNIN/Ob strain had 77% higher LPL activity in the RPWAT compared to their age and sex-matched lean control rats (AI) (**Figure**). Feeding of obese rats with 26, 52 and 129 mg of vitamin A (BII, BIII and BIV respectively) resulted in 36%, 34% and 27% decreased LPL activity compared to control diet-fed obese rats receiving 2.6mg of vitamin A (BI) (**Figure**). However, no change was observed in lean rats supplemented with various doses of vitamin A (AII, AIII and AIV) compared to control lean rats (AI).

CONCLUSION

The present study demonstrated the possibility that the diets supplemented with 52 and 129 mg of vitamin A/kg diet have anti-obesity and hypocholesterolemic effects in obese rats and there are no significant differences between these two groups. From these observations, we conclude that vitamin A when used at a dose of 52mg/kg diet is the minimum effective dose in bringing about reduction in weight gain and normalizing plasma cholesterol levels without inducing hypertriglyceridemia in these rats.

14 FLAVONOID CONTENT IN INDIAN FOODS

Legumes are important sources of protein, starch, dietary fiber, lipids and minerals. In addition to their nutritive value, legumes contain significant quantities of phenolic compounds such as phenolic acids and flavonoids. The legume that is attracting much attention recently, is the soybean, which has a high amounts of isoflavones, are a type of flavonoids with cardio-protective and anti-cancer properties.

The latter property's elicited due to their anti-estrogen activity. Although soybean products are not common in India, legumes such as green gram, chick and cow peas are staple foods in the diet of several populations. In addition to seeds, germinated sprouts of these legumes are consumed as much and used in soups, salads and side dishes. It is well known that the germination causes changes in the nutrient contents as well as the anti nutrients such as enzyme inhibitors and condensed tannins.

Variations in tannin and phenolic compound contents in germinated legumes have been reported by several authors. However data is lacking on variations in

flavonoid compounds specifically isoflavones in sprouts. In view of the health benefits of legumes and their phenolic compounds, it is important to generate data on the changes that occur due to processing mainly soaking and sprouting of commonly consumed legumes.

AIMS & OBJECTIVES

1. To study Flavonoid content in Indian foods.
2. To study the variation in flavonoid contents due to germination processing and seasonal changes.

Work done during the year 2008-2009

Three commonly consumed legumes such as green gram, chick and cow peas were analyzed for total phenolics and other flavonoids in soaked and germinated for 1 and 2 days.

METHODS

Samples of three legumes (Green gram, chick and cow peas) in triplicates were soaked at room temperature for 12 hours and germinated for 24 hours and 48 hours. The resulting sprouts were ground and extracted into 70% methanol in shaker water bath for 2 hours.

Total phenolic compounds were estimated by spectrophotometric method by using Folin and Ciocalteu's reagent.

Methanolic extracts were hydrolyzed with 2.0 M HCL at 90º C for 90 minutes and filtered through 0.45 µm and injected into HPLC.

HPLC-DAD Analysis for Isoflavones: Lichrosphere-ODS2, C18 column (250×4.6mm), was used for chromatographic separation on Shimadzu LC-2010A equipped with auto injector, binary pump and Diode array detector. Linear gradient of mobile phase 0.1% phosphoric acid (A) and acetonitrile (B) with a flow rate of 1ml/ min. The lineaer gradient was started at 10% acetonitrile and increased up to 100% and brought back to 10% in 35 minutes run time. A wavelength range of 200-800 was used in the DAD detector.

RESULTS

- Concentrations of phenolic compounds were different among the dried samples of three pulses. Cow pea contains the highest levels of total phenolics (Mean±SD 324.1±15.54 mg/100g) compared to chick pea (134.3± 9.65mg/100g) and green gram seeds 123.5 ±8.6565mg/100g.
- Soaking and germination differentially influenced the phenolic content of the three pulses.
- In green gram, the total phenolic content increased significantly after soaking ($p<0.05$) from 123.5 ±8.65 to 153.4±9.45mg/100g, and 24 hours germination time 179.6±13.25mg/100g. In contrast, the content of total phenolics decreased to120.6±7.43 mg/100g after 48 hours germination. Where as in cow pea, the total phenolic compounds reduced to >50%, 324.1±15.54 to 152.3 ±11.52mg/100g after soaking, slightly increased in sprouts germinated for 24 hours 144.0±8.95mg/100g and a drastic decrease was observed in 48 hours germinated seeds 69.4±7.45mg/100g.
- In chick pea, the total phenolic contents were not significantly different between the values dried134.3± 9.65mg/100g to soaked 125.1± 8.76mg/100g, and soaked to 24 hrs germinated seeds 144.0±11.23 mg/100g, whereas at 48 hrs germination point, the total phenolic content decreased significantly ($p<0.05$) 106.7±8.35 mg/100g compared to dried, soaked and at 24 hrs of germination.
- Total isoflavone contents significantly increased in the sprouts of chickpea seeds. However, no such change was observed in green gram and cow pea seeds.
- In chick pea seeds, total isoflavone contents were significantly increased ($p<0.001$) with 24 hrs and 48 hrs of germination (97.7±5.42µg/g, 619.35± 18.42µg/g respectively), compared to dried and soaked samples (34.0±2.25, 33.62±2.16µg/g). Biochannin A and formononetin are the predominant forms of isoflavones in germinated seeds and the

15 GENETIC POLYMORPHISM IN CANDIDATE GENE AND ITS ASSOCIATION WITH INSULIN RESISTANCE, CARDIOVASCULAR DISORDER AND HYPERTENSION

With the recent trend of rapid urbanization and industrialization, massive adoption of sedentary lifestyles, most of the developed and rapidly developing countries are facing a new epidemic now called as 'Metabolic Syndrome'. Clinically, metabolic syndrome is a constellation of many inter-related metabolic disorders including central obesity, increased low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C), increased triglycerides (TG), hypertension and hyperglycemia. This syndrome develops as a result of energy imbalance, which is often modified by genetic factors including family history and ethnicity. Genetic components make an individual susceptible to the disease and environmental factors such as life-style and age etc. act as trigger to the start of a full-blown disease. People with the metabolic syndrome face at least a three-fold and two-fold increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD), respectively. Similar to the variation in the prevalence of the individual components of the syndrome among populations, the prevalence of the metabolic syndrome also varies from one population to another greatly.

While several studies have been conducted on western populations, very few data are available on Indians. Present study is designed as a population based case-control study to understand the genetic make up of the Indian population *vis a vis* its predisposition towards metabolic syndrome and its individual components.

To estimate the contribution of genetic factors in the etiology of metabolic syndrome, a cohort of 699 unrelated subjects who presented in the Mediciti Hospital, Hyderabad, was investigated. Subjects were classified as type 2 diabetic (mean age 61.8$\pm$11.25 years) based on their past history and being on anti-diabetic or hypoglycemic drugs. Whereas, normoglycemic subjects who are over 50 years age (mean age 61.93$\pm$10.40 years) and without high glycosylated hemoglobin (7%) were included as controls. All the anthropometric and biochemical measurements were carried out in fasting condition. Weight, waist and hip circumferences were measured by standard methods. BMI was calculated by dividing weight (kg) by the square of height (m). Plasma triglyceride, total cholesterol and HDL-cholesterol were measured in duplicate by colorimetric enzymatic assay using kits supplied by BioSystems (Spain) following the manufacturer's instruction.

Genomic DNA was isolated from the blood samples and screened for four polymorphisms of adiponectin gene, one in TNF-α and one in resistin. Details of the polymorphisms along with their db-SNP ID and location are listed in Table 1. The amplicons were digested with appropriate restriction enzymes to determine the presence or absence of the variant alleles as mentioned in the Table 2. About 20 percent of the samples were randomly sequenced to further confirm the presence of the polymorphism.

For studying the adiponectin gene polymorphisms four major variants reported earlier for this gene namely, T+45G, C-11377G, G-11391A and G+276T in this population were choosen. In contrast to other studies, we were not able to detect these variants except T+45G in this study population. The genotype and allele frequencies of T+45G polymorphism in adiponectin gene were categorized according to T2DM, hypertension, two parameters of

Table1. List of polymorphisms and their chromosomal locations

S. No.	Polymorphism	Db SNP ID	Gene	Region	Location
1.	C-420G	rs1862513	*RETN*	Promoter	19p3.2
2.	G-308A	rs1800629	*TNF*?	Promote	6p21.3
3.	T+45G	rs2241766	*ADIPOQ*	Coding	3q27
4.	G+276T	rs17846868	*ADIPOQ*	Coding	3q27
5.	C-11377G	rs266729	*ADIPOQ*	Promoter	3q27
6.	G-11391A	rs17300539	*ADIPOQ*	Promoter	3q27

Table 2. Restriction enzymes, digestion temperatures and size of digestion product for the SNPs

S.No	Gene	Variation	Restriction Enzyme	Temp. (?C)	Amplicon size (bp)	product size(bp) (allele digested)
1	***Resistin***	C-420G	BbsI	37	533	329, 204 (wt)
2	***TNF***?	G-308A	NcoI	37	120	84, 36 (wt)
3	***Adiponectin***	T+45G	SmaI	25	430	268, 162 (wt)
4		G+276T	NsiI	37	269	234, 55 (wt)
5		C-11377G	HaeIII	37	250	215, 35 (wt)
6		G-11391A	HpaII	37	250	200, 50 (wt)

Table 3. Distribution of genotype and allele frequencies of T+45G polymorphism in *Adiponectin* gene according to clinical characteristics of the subjects

	T/T	T/G+G/G	MAF
Diabetes (p>0.05)			
Diabetic (348)	271 (77.8%)	77 (22.2%)	0.120
Non-Diabetic (348)	260 (74.7%)	88 (25.3%)	0.135
Hypertension (p>0.05)			
Hypertensives (350)	270 (77.1%)	80 (22.9%)	0.122
Normotensives (346)	261 (75.5%)	85 (24.5%)	0.132
Obesity (p>0.05)			
BMI ?23 kg/m^2 (450)	338 (75%)	112 (25%)	0.134
BMI <23 kg/m^2 (246)	193 (78.5%)	53 (21.5%)	0.115
High WC (456)	350 (76.8%)	106 (23.2%)	0.126
Low WC (240)	181 (75%)	59 (25%)	0.131
Met Synd (IDF) (p>0.05)			
MS (374)	289 (77.3%)	85 (22.7%)	0.122
Non-MS (322)	242 (75.2%)	80 (24.8%)	0.135
Met Synd (NCEP) (p>0.05)			
MS (449)	345 (76.8%)	104 (23.2%)	0.125
Non-MS (247)	186 (75.3%)	61 (24.7%)	0.134

Table 4. Distribution of genotype and allele frequencies of C-420G polymorphism in *Resistin* gene according to clinical characteristics of the subjects

	C/C	C/G+G/G	MAF
Diabetes			
Diabetic (350)	146 (41.7%)	204 (58.3%)	0.372
Non-Diabetic (348)	111 (31.9%)	237 (68.1%)	0.435
Genotype: OR- 1.53 (1.12-2.08) (p=0.0071*)			
Allele: OR- 1.53 (1.20-1.94) (p=0.017*)			
Hypertension			
Hypertensives (350)	132 (37.7%)	218 (62.3%)	0.400
Normotensives (348)	125 (35.9%)	223 (64.1%)	0.408
(p>0.05)			
Obesity			
BMI ?23 kg/m^2 (452)	168 (37.2%)	284 (62.8%)	0.404
BMI <23 kg/m^2 (246)	89 (36.2%)	157 (63.8%)	0.404
(p>0.05)			
High WC (458)	172 (37.6%)	286 (62.4%)	0.403
Low WC (240)	85 (35.4%)	155 (64.6%)	0.406
(p>0.05)			
Met Synd (IDF)			
MS (376)	141 (37.5%)	235 (62.5%)	0.408
Non-MS (240)	116 (36.0%)	206 (64.0%)	0.399
(p>0.05)			
Met Synd (NCEP)			
MS (451)	169 (37.5%)	286 (62.5%)	0.407
Non-MS (247)	88 (35.6%)	159 (64.4%)	0.399
(p>0.05)			

obesity (BMI and waist circumference) and metabolic syndrome (NCEP-ATP-III and IDF criteria) as shown in Table 3. It was found that the allele and genotype frequencies of T+45G polymorphism in adiponectin gene were not significantly different between the two genotype groups (T/T vs. X/G) in any of the categories. Study subjects were also analyzed for C-420G polymorphism in the resistin gene by PCR-RFLP exploiting a restriction site for BbsI near this variant. Clinical and biochemical profile of the subjects were categorized based on C/C and X/G genotypes of C-420G polymorphism. The genotype and allele frequencies of C-420G polymorphism showed a significant association with T2DM (OR (95%CI) 1.53 (1.12-2.08, p=0.0071) (Table 4). Serum resistin levels in different genotypes of the C-420G polymorphism were estimated by ELISA using kit from Adipogen Inc. Significantly higher levels of resistin were observed among C/G subjects as compared to

Figure 1. Serum resistin levels in C/C, C/G and G/G genotypes in overall population and subjects stratified based on type 2 diabetes and hypertension. Data represent as mean±SE

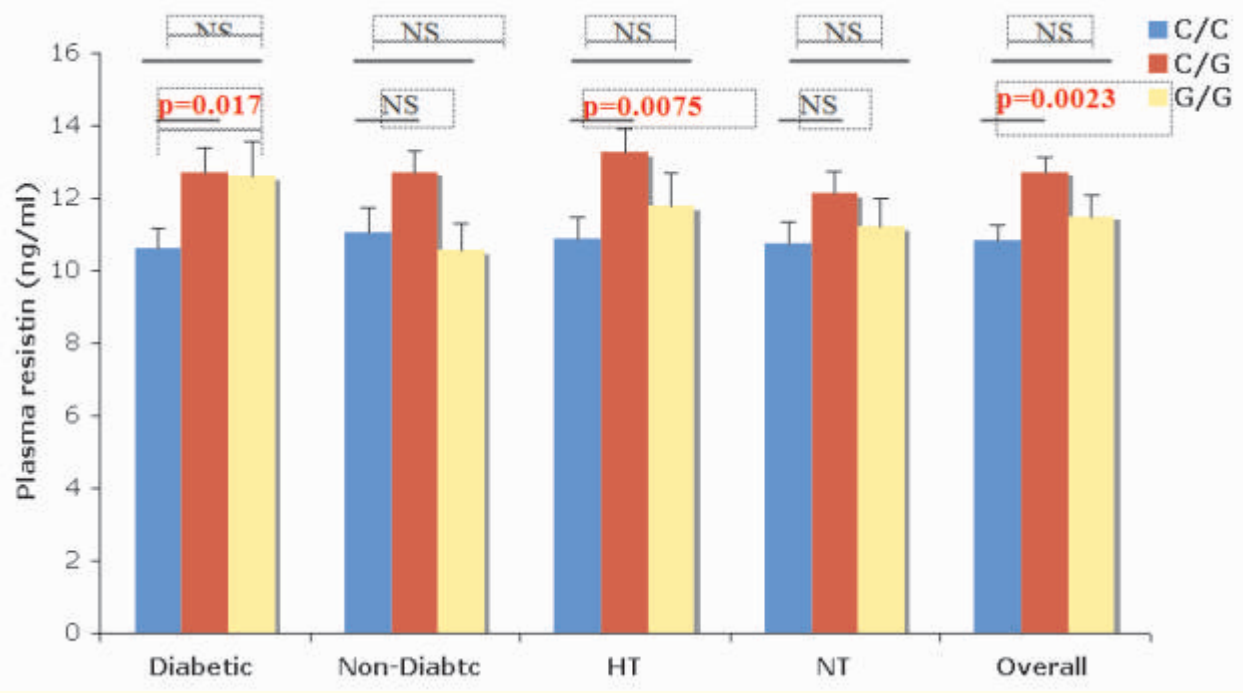
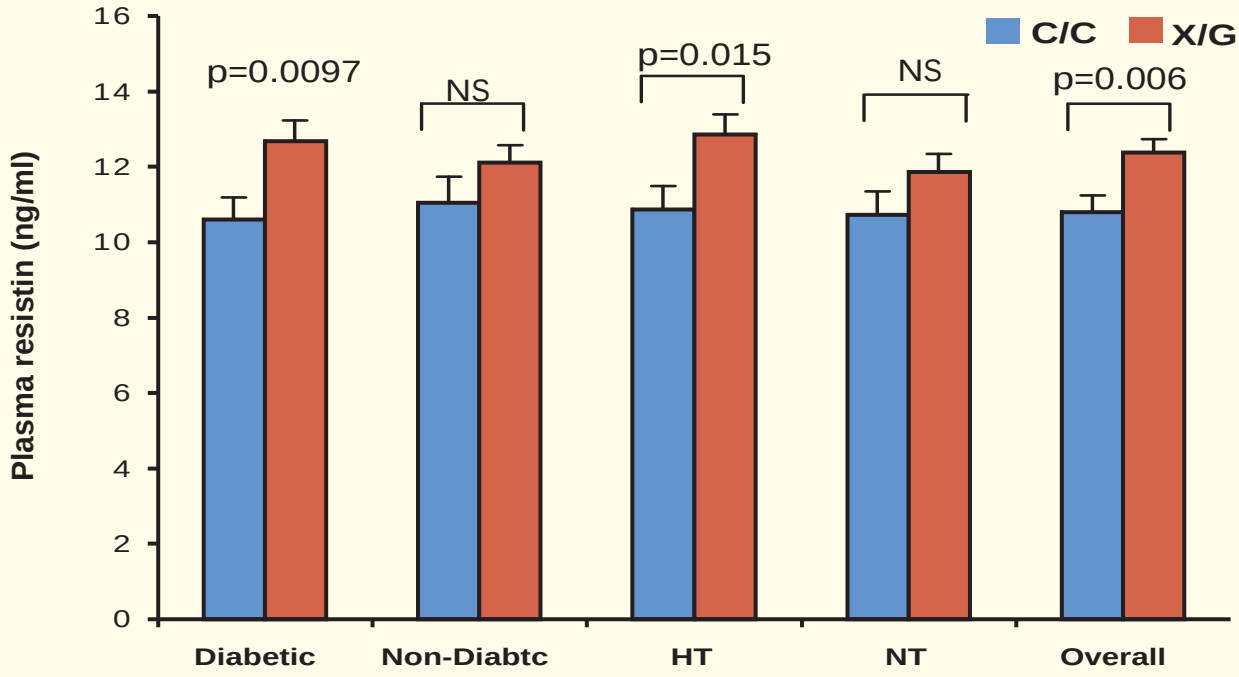


Figure 2. Serum resistin levels in C/C and C/G+G/G (X/G) genotypes in overall population and subjects stratified based on type 2 diabetes and hypertension. Data represent as mean±SE



subjects carrying C/C genotypes among diabetics (p=0.0173) and hypertensives (p=0.0075). However, the G/G genotypes did not show any significant difference in resistin levels (Figure 1). This could be due a low number of subjects carrying this genotype. Therefore, the subjects were reclassified as C/C and X/G genotypes. Significantly higher levels of plasma resistin were observed in carriers of G allele (X/G genotypes) as compared to homozygotes for C allele in diabetic (p=0.0097) and hypertensive (p=0.015) subjects, but there was no significant difference in resistin concentration between these genotypes among subjects categorized as non-diabetics and normotensives. Overall a significant association was observed in different genotypes of C-420G with resistin levels in overall subjects (p=0.006) (Figure 2). In summary, this study points to two important findings with regard to the relationship of C-420G with metabolic disorders. First, the G allele has some protective effect against T2DM in this population. Second, presence of G allele also causes an increase in plasma resistin concentration.

Interestingly, the G-308A SNP in TNF-α was found to be significantly associated with waist circumference (p=0.0125) but not with T2DM, hypertension and obesity. T+45G SNP in adiponectin gene was not associated with any of the disease conditions. Also surprisingly, we did not find three of the four variants of adiponecting namely, G+276T, G-11377A and C-11391G in this population. These data suggest a certain level of genetic uniqueness in Indian population and varied effects of different factors on different populations.

16 ENERGY REQUIREMENTS OF WOMEN ENGAGED IN DIFFERENT OCCUPATIONAL GROUPS

The accurate appraisal of energy needs of women engaged in different occupational groups based on their energy expenditure pattern is necessary as per the recommendations of FAO/WHO/UNU 1985 to recommend energy allowances for the maintenance of desirable body weight and composition for optimal work productivity.

OBJECTIVES

This study has been initiated to assess energy requirements of women from various occupational groups such as sedentary, moderate and heavy, through the following objectives.

1) To assess Basal Metabolic Rates (BMR),

2) To measure Energy cost of day-to-day physical activities, and

3) To evaluate daily energy expenditure levels through Time Allocation Pattern (TAP).

So far studies have been carried out on the women belonging to house makers, teaching profession, maidservants, sweepers, construction laborers/ unskilled laborers, agricultural laborers, and stonecutters representing sedentary, moderate, and heavy occupational groups.

During the current year a total of 45 apparently normal healthy, non-pregnant and non-lactating women, aged between 20 and 50 years, engaged in brick making activity who are representing moderate/ heavy occupational group were recruited. The subjects will be covered for 24-hour BMR, body composition, 24-hour Time Allocation Pattern (TAP), energy cost of standard activities, and 24-hour energy expenditure (in both occupational and non-occupational activities) in a phased manner. 30 women brick makers who had cooperated and participated in all the study parameters were

covered for the above said parameters. The anthropometry such as height, weight, and mid-arm circumference were measured using standard procedures. The skinfold thickness at four sites namely biceps, triceps, subscapular and suprailiac were measured with Holtain calipers.

Lean Body Mass (LBM) and Fat mass were derived from age and sex matched equations of Durnin and Womersley (1974). The resting metabolic rate (RMR), energy cost of standard activities like sitting, standing, and walking were measured by using Portable energy cost analyzer (Cortex-Meta Max-3B) based on open circuit indirect calorimetry. The 24-hour Time Allocation Pattern (TAP) was recorded and the time spent for the occupational and non-occupational hours was classified. The energy cost of occupational activities (brick making activities) and non-occupational activities were measured so as to evaluate 24-h energy expenditure.

The results of the study obtained so far are as follows:

1. The mean (SD) age of women was 28.67.81 years, height 151.94.14 cm and weight 42.45.38kg. The mean BMI (kg/m^2) and BSA (sqm) were 18.61.97 and 1.3460.086 respectively.
2. The body composition reveals that they had a mean LBM of 33.93.02kg and 8.43.27 kg fat mass. When considering the percent fat it was found to be 19.45.09.
3. The 24-hour Resting Metabolic Rate (RMR) was found to be 113095.1 kcal which was 27.14.29 kcal, when expressed in terms of unit (kg) body weight.
4. The parameters such as minute ventilation (MVE), heart rate (HR), RER, and energy cost of standard activities such as resting/lying, sitting, standing, and walking (without load) were measured and values are given in Table-1.

Table 1. Physiological Parameters during standard activities of women (Brick Makers)

Activity	MV_E (l/min)	HR(bpm)	RER	Energy Cost (Kcal/min)
RMR	4.45?0.88	78?6.07	0.94?0.012	0.785?0.661
Sitting	5.13?0.95	89?10.9	0.949?0.112	0.933?0.133
Standing	5.77?0.88	99?14.8	0.989?.0.080	1.141?0.174
Walking	11.66?1.81	105?14.6	0.829?0.085	2.964?0.629

Activity	Duration (min)	Energy Expenditure (K.Cal/day)
Occupational	421.67? 38.61	1549.74? 224.15
Non-Occupational	1018.33? 38.61	1115.32 ? 81.03
Total	**1440**	**2665.06**? **226.36**

5. The data pertaining to Time Allocation Pattern of various day-to-day activities in both occupational and non occupational hours and corresponding energy cost of activities of the subjects which gives the total daily energy expenditure revealed that the brick makers were spending about 2665226.3k.cal (Table 2).

The results of the study reveal that the women engaged in brick making can be classified under moderate occupational group based on their total daily energy expenditure levels (2665k.cal) and BMR factor of 2.35.

IV. EXTENSION AND TRAINING

A. SERVICE ACTIVITIES

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 are being published.

The other titles which were reprinted, on popular demand include Recommended Dietary Allowances, Nutrition for Mother and Child (Telugu), Diet and Diabetes (English).

2 TRAINING PROGRAMMES

Regular Training Programmes: This year a total of Nineteen candidates have attended the regular training programmes of the Institute viz. (i) Post-Graduate Certificate Course in Nutrition (12 participants including two candidates sponsored by WHO) (ii) Annual Training Course in Endocrinological Techniques (7 participants).

Apart from the above, efforts were intensified to commence the regular MSc (Applied Nutrition) Course from the academic year 2009-10. In place of the earlier 9-month MSc (AN) Course, the current programme has been converted into a 2-year regular Course with an affiliation to Dr.NTR University of Health Sciences (NTRUHS), AP. Candidates with bachelors degree in Medicine, Nutrition, Home Science, Bio-chemistry and Nursing will be eligible to apply for the Course. The regulations, curriculum and other details have been finalized in consultation with Dr.NTRUHS. The admission notification will be issued in May 2009 and other admission procedures will be completed in June-July 2009. The Course will Commence in August 2009. For the first batch the intake will be restricted to 16 students and it will be scale up to the full capacity of 32 by the next academic year.

3 EXTENSION ACTIVITIES

3.1. Exhibitions

* Participated in the exhibition organized by Press Information Bureau as part of Bharat Nirman Public Information Campaign, at Gajwel, Medak District. Posters were displayed on importance of Health and Nutrition in Telugu and English languages and delivered talks on “Importance of nutrition” during one of the sessions. (Sept.7-11)

* Participated in the Medical Exhibition “MEDEX”, organized by Osmania Medical College, Hyderabad. Posters on “Health and Nutrition” were displayed at a stall in the medical exhibition. (Nov. 20 – 30)

* Organised a Nutrition stall in association with Department of Public Health & Information & Public Relations, Govt. of Andhra Pradesh during the All India Industrial Exhibition, at Hyderabad. (Jan 1. – Feb.15)

3.2. Popular Lectures/Awareness Camps

* A series of Nutrition awareness programmes for adolescence and youth were conducted at summer camps organized by an NGO– Confederation of Voluntary Associa-tions. (May 2008).

* Participated in Awareness programme for Anganwadi workers, ICDS functionaries and rural women, at Chilkruru, Rangareddy District, Andhra Pradesh, organized by Department of Women Development and Child Welfare, Government of Andhra pradesh. (Sept. 1)

* An extension lecture was delivered on Nutrition for NSS volunteers of

Government PG and Degree College for Women, Jugdish huts slum area of old city of Hyderabad. (Dec. 29)

* Delivered a lecture on Nutrition awareness at Public Health Awareness Programme on "Nutrition, Sanitation and Personal Hygiene", for residents of Railway Colony, organized at Railway Institute, Moulali, Hyderabad. (Jan. 7)

* Delivered lectures on "Vector borne diseases" and extension lecture on "Nutrition" to the Medical officers of Primary Health Centers, Govt. of Andhra Pradesh, at the Institute of Health and Family Welfare, Hyderabad. (Jan.31)

* Participated and delivered the lectures on "Nutrition and Health", "Nutrition communication and Dietary guidelines" and "Food Safety", in a two day National Level Workshop on "Nutritional Awareness – Role of Educational Institutions", Organised by Govt. College of Arts and Science, Goa. (Feb. 20-23)

3.3. Radio talks

* As part of World Breastfeeding Week celebrations, a radio talk on "Importance of Breastfeeding", was delivered in Telugu language.

4 SPECIAL EVENTS

National Nutrition Week Celebrations (Sep 1st – 7th, 2008)

* A popular article on "Low cost nutritious supplements" was published in the Eenadu Telugu daily. (Sept.1).

* In collaboration with Department of Women's Development and Child Welfare, Govt. of Andhra Pradesh, Scientists of the Extension and Training Division participated in the Programme of Dissemination of Nutrition related information in five different districts of Andhra Pradesh. (Sept.1-Sept.6).

5 ACTIVITIES OF SECRETARIAT FOR WHOSEA NUTRITION RESEARCH-CUM-ACTION NETWORK

The Extension and Training Division has been carrying out the activities of the Secretariat for WHO Southeast Asia Nutrition Research-Cum-Action Network since 2004. As part of the day-to-day activities, correspondence related to the Secretariat of the WHO South East Asia Nutrition Research-cum-Action Network was carried out. Two issues of the half-yearly newsletter 'SEA NUTRITION' have been brought out and circulated to all the network members in 11 south east Asian Countries.

The Ninth meeting of the South East Asia Nutrition Research-cum-Action Network was held during 24th to 26th September 2008 at the National Institute of Nutrition, Hyderabad, India. The meeting was organized by the South-East Asia Regional Office of the World Health Organization (SEARO) and the SEA Nutrition Research-cum-Action Network Secretariat, National Institute of Nutrition, India.

All the eleven Member States of SEARO viz., Bangladesh, Bhutan, DPR Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste - participated in the meeting. In addition, the regional and national representatives from FAO, UNICEF, United Nations World Food Programme (WFP), the International Council for the Control of Iodine Deficiency Disorders (ICCIDD), the International Centre for Diarrhoeal Diseases Research – Bangladesh (IDRR,B), the Micronutrient Initiative (MI), the United States Agency for International Development (USAID) and a number of select experts participated in the meeting. The objective of the meeting was to review the current status of the SEA RCA Network and its relevance to the National Nutrition Policies and Plans of Action; and to assess the rising food insecurity and its implications on the health and nutritional status of the vulnerable

populations and identify appropriate action plans.

Appreciating the South-East Asia (SEA) Nutrition Research-cum-Action (RCA) Network, which is one of its kind and unique to this region, the meeting felt that the Network should be further strengthened in the areas of operation research and wider dissemination and exchange of technical information among the Member States, academic institutions and interested national and international partners.

The meeting also recommended that comprehensive behaviour change communication strategies should be evolved at the country level in order to address the problems of all forms of malnutrition. For this, the experiences of ongoing efforts should be shared among member countries.

B. RESEARCH ACTIVITIES

1 A study on approaches to nutrition communication

Nutrition Communication and the resultant positive result, if any, are difficult to reproduce routinely on a large scale. Given the limited resources available in most settings, nutrition communication efforts are usually designed to have an impact on large sections of the population in a cost-effective way. The following are important for affordability, effectiveness, and reach, particularly for large-scale programmes (Smith et al, 1998) - programme design, targeting, duration, community participation, and strategies to support environments to strengthen local ownership and to develop structural and institutional support.

There are many players in the field of nutrition communication, with a variety of programmes aimed at larger audience. Today nutrition communication is part of many a development and health programmes across the sectors. As the National Nutrition Policy, 1993 recognizes "...nutrition affects development as much as development affects nutrition...", nutritional concerns are being integrated into various developmental policies and programmes being taken up at various levels by the Government. Non-Governmental Organisations (NGOs), international organisations and the research institutes are also putting in considerable efforts in taking the message of nutrition to the community. It is a common understanding that in different nutrition communication programmes by different organizations, the extent of participation varies and accordingly the model adopted is likely to differ. A study was conducted with the following aims:

1. To document nutrition communication approaches being adapted by three different organizations in three different sectors (one each from Government Department and Research and Development)
2. To understand the notion of nutrition communication as perceived by these organizations (or the implementers in the organizations)

METHODOLOGY

'Case study method' was employed for the study. The 'case study' is a research strategy which focuses on understanding the dynamics present with single setting while studying the particularity and complexity of a single case covering its activity within important circumstances *(Eisenhardt, 2002; Stake, 1995).*

For the present study, three different organizations from four different sectors were purposively selected for 'case studies'. The Food and Nutrition Board of Government of

India was selected from the Government Sector, National Institute of Nutrition (NIN) from Research and Development Sector and the Deccan Development Society (DDS) from the Voluntary Sector.

The case studies typically combined data collection methods such as information gathering from archives, in-depth interviews with the key people involved in nutrition communication in each organization and observations (wherever possible). Semi-structured in-depth interviews were also conducted with the key people involved in nutrition communication. In preparation for the semi-structured in-depth interviews, a theme guide was prepared. The theme guide listed the topics around which the interviews would focus, viz., nutrition communication activities of the organization and target audience; Planning for nutrition education and communication and monitoring and evaluation; budget; indicators for success of the nutrition communication efforts – laid out in the programme and/or perception; community participation

For summarizing each of the organizational case studies, a standardized format (Smith, 1998) was used. Each case study summary was followed by brief discussion, which provides conclusions regarding the trends which emerge overall, how these compare with past reviews and what examples of best practice are provided by these studies to better inform similar projects in the future.

RESULTS

Case Study-1

Food and Nutrition Board: The Food and Nutrition Board (FNB) was established in the Department of Food, Ministry of Agriculture in 1964 as a non-statutory ministerial wing with the objective of diversifying Indian diet for improving the nutritional status of the people. The functions of the Board included development and popularization of subsidiary and protective foods; nutrition education; extension and food management; conservation and efficient utilization of food resources; and food preservation and processing. After the Government of India adopted the National Nutrition Policy in 1993, FNB was transferred to the Department of Women and Child Development. The infrastructure of the FNB comprises of a technical wing at the Centre, four regional offices and quality control laboratories at Delhi, Mumbai, Kolkatta and Chennai and 43 Community Food Nutrition and Extension Units (CFNEUs) located in 29 States and UTs. The major activities of the Food and Nutrition board are - nutrition education and training, training of home scale preservation of fruits and vegetables and nutrition, monitoring of 'Supplementary Feeding' and 'Nutrition and Health Education' components of the Integrated Child Developmet Services (ICDS); mass awareness campaigns, mass media campaigns; development, production and distribution of educational/training material; food analysis and standardization.

Inferences from the qualitative study involving in-depth interviews with the key people involved in the nutrition education and communication activities revealed that in training of the functionaries or training of the community or reaching the message of nutrition to the 'beneficiaries' through them, the organization's approach appears to be didactic. Hence it can be summarized as 'top-down' approach with some emphasis on multiple-step flow of nutrition communication.

This model appears to be largely shrouded in a way in the 'opinion leader theory' of Katz and Lazarsfeld (1955), which postulated that interpersonal communication plays a crucial role in channelling and shaping the opinion. There are two or more steps in information flow viz., from the source to the opinion leaders, and from leaders to the 'masses'.

Considering that the approach(es) chosen by FNB for communication of nutrition

information is dominated by conventional educational approaches that emphasize knowledge transmission and acquisition with an inherent assumption that these would ultimately lead to change in attitudes or behaviours, it can even be categorized under the 'Information dissemination' approach of nutrition communication indicated by Valyasevi and Attig (1994). As there is intermittent use of mass media and additional educational material like posters, mass media etc., it partly adheres to the 'Education Communication' model as well. Over all, the case study brought to the fore, a number of issues like lack of systematic evaluation of the programme on the whole and the communication (rather education) component in specific, complete lack of planned communication effort and an almost elusive feedback mechanism. These make it impossible to assess the role of FNB's nutrition communication programmes in achieving nutritional improvements on a national scale. Furthermore, lack of knowledge of the staff on the importance of 'learning from the community' before 'making the community learn' coupled with large number of target programmes that they have to conduct and limited resources (both financial and infrastructure) allocation underline the need for a thorough re-look at the approaches employed for nutrition communication.

Case Study -2

Deccan Development Society(DDS): The Deccan Development Society (DDS) is a Non-Government Organisation (NGO) based in Zaheerabad area of Medak district in the southern state of Andhra Pradesh, India. Incorporated in 1983, this grassroots organisation is working in over 75 villages with women's societies. With over 5000 registered members, the Society aims to bring the village groups (*Sangams*) together into a strong pressure group for women, *dalits* (socially marginalized) and the poor, and to facilitate debate, discussion and educational activities that will encourage local governance and autonomy over local resources. Listed below are the various initiates the NGO is working on – building autonomy over food production, autonomy over seeds, autonomy over natural resources autonomous market and autonomous media. They successfully got control over their own food production, seeds, natural resources, healthcare systems, markets and media.

Since 1995, DDS *sangams* have been running an 'alternative public distribution system' in over 50 villages. This is a self-provisioning food system based on the principles of local production of local foods, local storage and local distribution. By bringing cultivable fallow land under production, the women have been producing a basket of crops through a biodiversity-based, ecological food-production system. The focus was on knowledge-based farming, which underlines the importance of mixed cropping, cultivation of native varieties of grains and millets, soil and water conservation, organic agriculture etc. Promoting food sovereignty being one of the important efforts of DDS, the local population has been working towards taking control over their food sovereignty by promoting village-level Community Gene Funds. The women map the surrounding villages to gauge families' entitlements depending on their levels of poverty. Instead of queuing up to plead with the government officials for their ration entitlements, the villagers have almost reached a position to control grain and its distribution. DDS has also been working towards ensuring household food security of the poor and the marginalized by encouraging them to work collectively on their marginalized lands towards its incremental upgradation.

All the initiatives of the society are run through - Participatory Rural Appraisal [PRA]. This methodology ensures, all the programs will have total participation of the community,

especially the women. The society only acts as a catalyst to guide the community into those activities that the community decides to pursue. The qualitative study revealed that although, food and nutrition related communication is not exclusively dealt with, it is an integral part of information exchange. Especially in efforts related to reviving of traditional food habits, millets and less known greens that were once very popular in the region (before populist rice, wheat distribution schemes of the Government PDS), the NGO had in fact learnt from the community and validated the traditional knowledge with the scientific evidence before popularizing the traditional knowledge in the community.

At the same time the participatory approach leading to empowerment of women and socially marginalized groups, safeguarding their food sovereignty and creating market autonomy through alternative public distribution system all contribute to the holistic approach to communication. However, nutrition communication by itself does not figure as a special feature in any of the DDS' activities. Monitoring and evaluation of the food and nutrition related activities (along side others), although managed by the community itself, have been found to be lacking strong evidence to show that the communication efforts have been successful in bringing about health benefits.

Case Study -3

National Institute of Nutrition: The institute is India's premier research institute in the field of Nutrition, working under the Department of Health Research, Indian Council of Medical Research, Ministry of Health and Family Welfare, Government of India. Institute's activities are broad-based, encompassing the whole area of food and nutrition. The Institute has achieved close integration in its research activities between the laboratory, the clinic and the community. The Extension and Training Division, which was started in late 1960s is mandated with the tasks of nutrition education, communication, and capacity building and information dissemination. As regards the nutrition communication activities, the Department of the Institute that spearheads this activity is the 'Extension and Training Division'. The Division's activities are multi-pronged encompassing, Nutrition Communication Research, Human Resource Development, Information Dissemination (by way of exhibitions, awareness programmes, popular publications etc).

Over all analysis of the most communication or education efforts of NIN appear to be dabbling in away in the 'communication–effects' perspective with an implicit assumption that isolated individuals are relevant behavioural units. Many communication efforts of NIN seem to be viewing the effects (in terms of knowledge gain or behaviour adoption) from the 'sender's' perspective and do not seem to have examined the unintended consequences of communication especially given the fact that the individuals are not atomized units unconnected and uninfluenced by the context.

The other term that repeatedly occurred during in-depth interviews, was the Information, Education and Communication (IEC) approach. There are three underlying components that seem to be promoting awareness and understanding of nutrition issues among the 'common people'. Firstly, by providing information i.e., facts and issues to the attention of audience in order to stimulate discussion in extension and awareness programmes. Next, the education component which aims to foster knowledge and thorough understanding of problems and possible solutions through formal and non-formal education sub-components aimed at strengthening human resources by training. Finally, the 'communication' component by way of smaller research efforts, with an ambition to influence attitudes, disseminate knowledge and to bring about a desired and voluntary change in behaviour. The nutrition

education/communication programmes are still grounded in the 'extension' mode of activities and do not seem to address issues beyond mere sensitization or awareness creation. The larger perspective of understanding the context of the audience and advocating for creation of enabling environment for bringing about the desired behaviour change seem to be lacking. Since capacity building and extension activities use inter-personal communication, face-to-face communication coupled with other communication tools such as posters, brouchers, flip charts and films, they can be broadly characterized under the 'Education Communication Approach' mentioned by Valyasevi and Attig (1994). To sum up it appears that the selection of specific communication approaches is not primarily based on their analytical or normative value, but rather, on institutional factors and expectations.

CONCLUSIONS

The three case studies presented different institutional perspectives on nutrition communication. Although nutrition communication is one of the primary activities of FNB and NIN, the methods adopted are predominantly rooted in top-down approaches with information dissemination as an important objective. The capacity building and community oriented extension activities of NIN or the awareness programmes and demonstrations of FNB largely adopt inter-personal or face-to-face communication aided with a range of small media (like folders, charts, folk dance forms in local languages). Many researchers have concluded that inter-personal or face-to-face communication is widely used and plays a key role in health communication. Although DDS adopts participatory approach, its primary focus is not food and nutrition. The organisation views food and nutrition communication as a tool to engage women in achieving food autonomy and towards attaining the broader goal of women empowerment. Its food and nutrition communication efforts are largely concentrated in reviving the traditional agriculture practices and thereby the traditional food habits in the region. The model truly meets the participatory approach of communication. But this evidence is barely enough to conclude its effectiveness on a broader canvas. Yet another important observation is that the communication activities of all the three organizations have not been according importance to the evaluation component while planning the nutrition communication. This makes it difficult to attribute any change be it in behaviour or in improvement of nutritional status to a particular communication process. It is the institutional goals and dynamics and budgetary constraints and not the normative values that determine the use of the models or approaches to communication.

2 Coverage of nutrition related topics by print media : A comparative analysis of leading English and Telugu dailies in Hyderabad, India

Newspapers play a vital role in disseminating knowledge on various aspects of information relevant to the community. The reporting of news about medicine, public health, and nutrition is an area of concern to many scientists. Media coverage, including views and content expressed on editorial pages can affect health aspects, both positively and negatively. It is not uncommon to find some of the reports on nutrition published in print media lack scientific basis or acceptable information. News media over-and under emphasize certain causes of ill-health for a variety of reasons, including competition for viewers and commercial interests. Often, this contributes to confusion among the readers in addition to misinformation. Hence, a study was conducted on health related

topics with special reference to nutrition in leading English and Telugu dailies published from Hyderabad, India, in terms of acceptability or scientific validity of the information.

OBJECTIVES

a) To assess coverage of nutrition related topics for a period of six months by leading English and Telugu newspapers published from Hyderabad, India, and

(b) To comparatively analyze between English and Telugu language dailies in terms of number of articles, visuals and priority given in space allocation for nutrition related topics.

METHODOLOGY

Nutrition Related **(NUTRE)** topic is defined as "any information (excluding advertisements) appearing in newspapers which enables its readers to derive benefits in terms of nutrition if they practice/ follow the suggestions." The word "article" used in this paper denotes inclusion of news items, feature articles, tips, interviews, research/ survey findings, recipes (with emphasis on nutrition), opinion columns and editorials. Articles containing words related to nutrition, proteins, vitamins, minerals, food, diet, etc were considered as NUTRE articles. Based on the circulation figures six leading newspapers (three from each language) were selected for this study. Accordingly, English dailies-Deccan Chronicle, The Hindu, Times of India and Telugu dailies-Eenadu, Andhra Jyothi and Andhra Bhumi were taken for analysis. Each newspaper was screened everyday for a period of six months i.e., from 1st September 2007 to 29th February 2008. During the study period (excluding festival holidays as the newspapers do not get published) 179 copies of each newspaper amounting to a total of 1,074 copies were screened for NUTRE information.

For the purpose of assessment, grades were assigned to each NUTRE article based on the priority of their appearance or style of presentation and degree of possibility to grab readers' attention. Higher grade 'A' with a score of 4 is attributed to each NUTRE article appeared prominently and lower grade 'D' with only one score is given to the items appeared with less importance (Table 1). For the purpose of comparative analysis, the following 16 topics related to nutrition appeared in the newspapers were broadly identified:

Table 1

1. Cereals/ Pulses/ Millets
2. Oils/ fats/ nuts
3. Milk/ egg/ meat/fish
4. Fruits & Vegetables
5. Vitamins & minerals
6. Balanced diet
7. Seasonal foods
8. Chocolates/ Ice-creams
9. Beverages
10. Child nutrition
11. Overweight /obesity
12. Diet to retain beauty
13. Food additives
14. Junk food
15. Diet to fight diseases
16. Others

STATISTICAL ANALYSIS

Four experts in the field of nutrition have rated the NUTRE articles based on the above parameters. Cohen's kappa statistical test has been used to measure inter-rater agreement and found that there is a significant consistency between Rater 1 Vs Rater 2 Vs Rater 3 Vs Rater 4 and *vice versa* ($P<0.01$). Categorical classification was used to assess reliability of agreement between the multiple raters and observed that the intra-class correlation was more than 0.9 indicating existence of significant correlation ($P<0.01$) between all the four raters.

RESULTS & DISCUSSION

A total of 667 NUTRE articles (Table 2) appeared in the six dailies *viz.* Deccan Chronicle, The Hindu, Times of India, Eenadu, Andhra Jyothi and Andhra Bhumi during the study period. Overall, Telugu newspapers, Eenadu, Andra Jyothi and Andhra Bhumi combinedly covered more number of articles (345) than Englsih group of dailies (322). In all three Telugu dailies, articles on nutrition, mostly appeared in Health Page or in Women's page indicating as these articles were meant for target readers (Figure 1). About 66% of NUTRE articles in Eenadu and more than 75% in Andhra Jyothi appeared either in Health Page or in Women's Page. In Andhra Bhumi, except one, all NUTRE articles appeared only in Women's Page as if, it is a subject of feminine. The Hindu and Times of India presented significant number of NUTRE articles in their Sunday magazine section. Usually, any article if appears in a daily's Sunday magazine section enjoys privilege of largest readership than normal days. All three Telugu dailies published excess number of visuals than the articles produced. A total of 278 visuals published only in Eenadu against 170 articles on NUTRE topics, followed by Andhra Jyothi with 179 photos against 133 articles. Andhra Bhumi, though figured less NUTRE articles (42), published 67 photos, which is 60% in excess. On an average, Telugu newspapers presented about two visuals per each article. It is rare to find NUTRE articles appeared in Telugu dailies without a visual. Particularly in Eenadu and Andhra Jyothi, even single column message on nutrition consists suitable photo.

Based on the grade given to each article, Eenadu gained a score of 405 and stood first rank followed by Andhra Jyothi with a score of 352, Deccan Chronicle with 309, The Hindu with 294, Times of India with 262 and Andhra Bhumi with only a score of 94 stood last in the list (Table 3). Contrary to the total number of articles, all the three English dailies as a group scored higher points (865) than the group of Telugu newspapers (851) in coverage of NUTRE articles. To elucidate, English dailies though published less number of NUTRE articles was able to score more points compared to their counterpart Telugu newspapers. This indicates that, English dailies have demonstrated NUTRE articles a bit more prominently than Telugu newspapers. English dailies even maintained a balance in presentation of NUTRE articles under different grades. The Hindu and Times of India covered around 50% of the NUTRE articles under grade B and remaining 50% of the articles in grades A and C almost equally. The Hindu and Times of India published significant number of NUTRE articles in grade A, whereas NUTRE articles in Telugu dailies lack this prominence. Moreover, significant no. of NUTRE articles in Telugu dailies appeared in grade D. Eenadu and Andhra Jyothi published majority articles in grade B and C. NUTRE articles under grade D are insignificant in The Hindu and Times of India.

Overall, Fruits & Vegetables topic was widely covered and most frequently appeared in all the daily papers in the study (Table 4). Topic-wise, 'fruits & vegetables' gained first rank (176 articles) and 'overweight / obesity' occupied distant second place with 75 articles. This phenomenon was displayed in all the newspapers uniformly irrespective of their circulation figures and language of the media. On an average, out of every four NUTRE articles appeared in newspaper, one belongs to the topic of fruits & vegetables. Among NUTRE articles, coverage of overweight/ obesity topic was more in English dailies compared to Telugu newspapers. Percentage of overweight / obesity articles appeared in the three English dailies combine was 14% against mere 8% articles on this topic in the Telugu dailies trio. A clear variation was evident between English and Telugu dailies in terms of percentage of each nutrition topic they covered. Telugu dailies published more percentage of articles on balanced diet and

Table 1. Parameters for grading nutrition related articles

Grade A Score @ 4	Grade B Score @ 3	Grade C Score @ 2	Grade D Score @1
NUTRE topics appeared in	NUTRE article:	NUTRE article:	NUTRE article:
1. Front page of the newspaper's main page / city tabloid or	1. Description with more than one visual /graph / table put together or	1.Description with only one visual occupied less than 1/5th space of the page or	1. Description of only a single column without any visual or
2. Sunday magazine or other special supplements cover story or	2. Occupied more than 1/5th space of the page including visual or	2. Only a visual with caption, no description or	2. Consist only a visual without proper caption/ description or
3.Inside Sunday magazine with at least one visual or	3. Highlighted to prevent/fight or caution against diseases/ obesity/fatigue/ageing or	3. A mention in an article on health or other subjects or	3. Though content have scientific basis, it is not applicable/ practicable to Indian context.
4 (i) Editorial page or (ii) Letters to the Editor column	4. (i)Highlighted a single nutritive rich food or caution about harmful foods (ii) Highlighted to caution about harmful foods.	4. Covered as an event / Research or survey reports / Informative.	4. Tips to enhance nutritive values of food items

Note: If any article qualified under more than one grade, the higher grade was assigned to that article

Table 2. Cumulative statement of nutrition related articles appeared between 1st Sept. 2007 and 29th Feb. 2008

	Deccan Chronicle	The Hindu	Times of India	Eenadu	Andhra Jyothi	Andhra Bhumi	Total
No. of nutrition articles	128	101	93	170	133	42	667
No. of words	33,231	33,767	25,003	31,464	26,886	20,901	1,71,252
No. of visuals	99	89	92	278	179	67	804
Special or Sunday magazine	3	18	14	9	1	0	45
Health page	64	20	34	39	51	0	208
Women's page	NA	NA	NA	72	50	41	163
First page	5	1	2	1	3	0	12

NA = Not applicable

Figure 1. Percentage of NUTRE articles appeared in exclusive pages

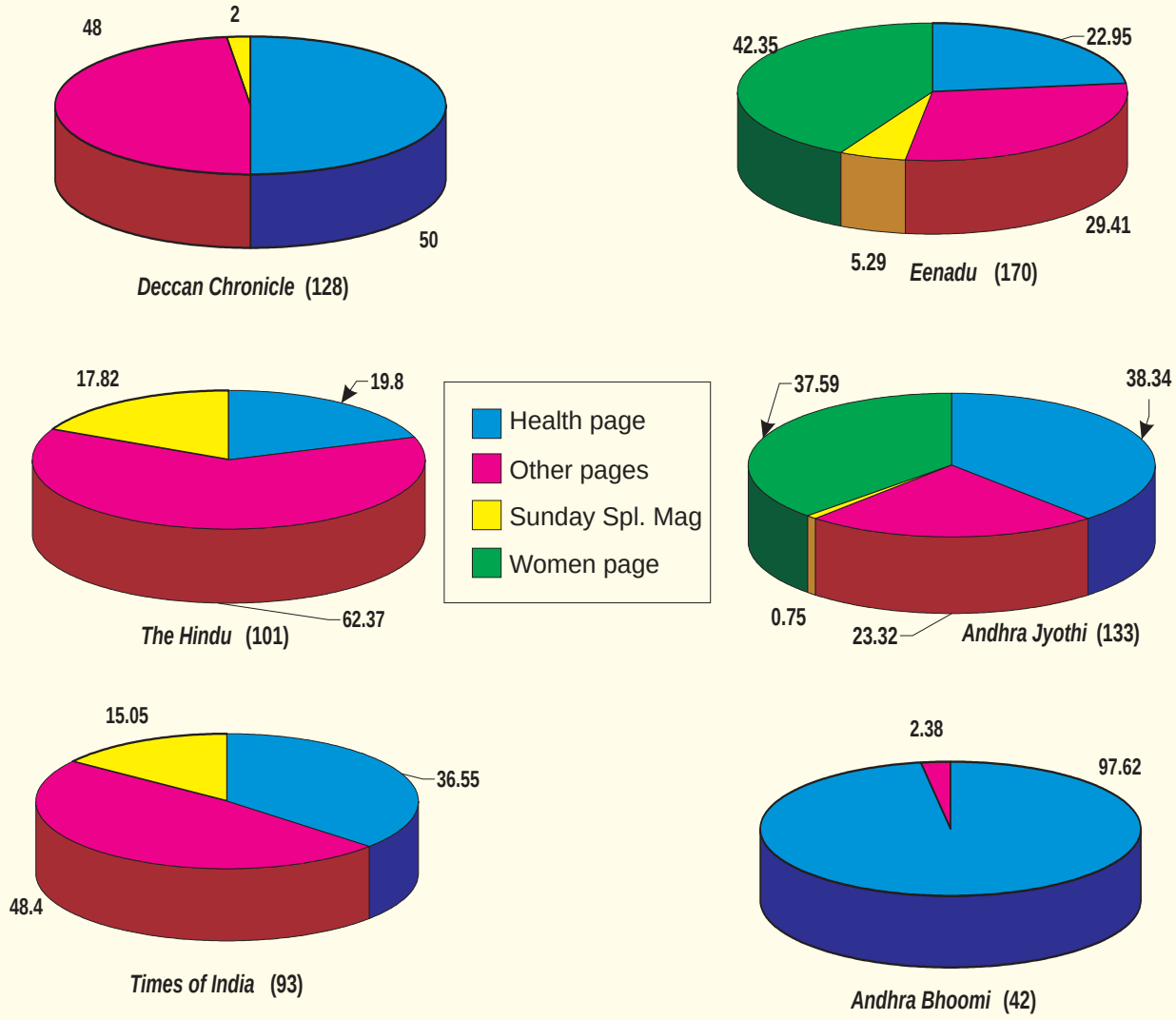


Table 3. Scores assigned to NUTRE articles based on the grade they qualified

	Deccan Chronicle		The Hindu		Times of India		Eenadu		Andhra Jyothi		Andhra Bhoomi	
	No. of articles	Total Score	No. of articles	Total Score	No. of articles	Total Score	No. of articles	Total Score	No. of articles	Total Score	No. of articles	Total score
Grade A Score @ 4	9	36	21	84	20	80	8	32	4	16	1	4
Grade B Score @ 3	62	186	53	159	42	126	77	231	90	270	14	42
Grade C Score @ 2	34	68	24	48	25	50	57	114	27	54	21	42
Grade D Score @ 1	23	23	3	3	6	6	28	28	12	12	6	6
Total	**128**	**313**	**101**	**294**	**93**	**262**	**170**	**405**	**133**	**352**	**42**	**94**

Table 4. Topic-wise focus of nutrition related articles appeared in leading Newspapers from 1st Sept. 2007 to 29th Feb. 2008

S.No.		Deccan Chronicle	The Hindu	Times of India	Eenadu	Andhra Jyothi	Andhra Bhumi	Total
1	Cereals, Pulses, Millets	*3* **2.34**	*7* **6.93**	*4* **4.30**	*12* **7.06**	*8* **6.01**	*2* **4.76**	*36* **5.40**
2	Oils, fats, nuts	*9* **7.03**	*8* **7.92**	*2* **2.15**	*16* **9.41**	*7* **5.26**	*6* **14.30**	*48* **7.20**
3	Milk products, egg, meat, fish	*12* **9.38**	*1* **0.99**	*5* **5.37**	*10* **5.90**	*4* **3.01**	*3* **7.14**	*35* **5.25**
4	Fruits and vegetables	*29* **22.65**	*28* **27.73**	*16* **17.20**	*45* **26.47**	*42* **31.58**	*16* **38.10**	*176* **26.38**
5	Vitamins and minerals	*6* **4.69**	*5* **4.95**	*7* **7.53**	*8* **4.70**	*10* **7.52**	*1* **2.38**	*37* **5.55**
6	Balanced diet	*4* **3.12**	*6* **5.94**	*12* **12.90**	*19* **11.18**	*12* **9.02**	*3* **7.14**	*56* **8.39**
7	Seasonal foods	*1* **0.78**	*1* **0.99**	*2* **2.15**	*5* **2.94**	*3* **2.26**	0	*12* **1.80**
8	Chocolates, Ice-creams	*9* **7.03**	*4* **3.96**	*8* **8.60**	*11* **6.47**	*5* **3.76**	0	*37* **5.55**
9	Beverages	*12* **9.38**	*2* **1.98**	*6* **6.45**	*3* **1.76**	*4* **3.01**	*1* **2.38**	*28* **4.20**
10	Child nutrition	*2* **1.56**	*3* **2.97**	*5* **5.38**	*5* **2.94**	*4* **3.01**	*2* **4.76**	*21* **3.15**
11	Overweight/ obesity	*15* **11.72**	*17* **16.83**	*13* **13.98**	*14* **8.23**	*13* **9.77**	*3* **7.14**	*75* **11.24**
12	Diet to retain beauty	*8* **6.25**	*3* **2.97**	*5* **5.38**	*11* **6.47**	*7* **5.26**	*1* **2.38**	*35* **5.25**
13	Food additives	*6* **4.69**	*3* **2.97**	*1* **1.08**	*3* **1.76**	*3* **2.26**	*2* **4.76**	*18* **2.70**
14	Junk food	*2* **1.56**	*3* **2.97**	*2* **2.15**	*1* **0.59**	*4* **3.01**	0	*12* **1.80**
15	Diet to fight diseases	*5* **3.91**	*3* **2.97**	*3* **3.23**	*2* **1.18**	*5* **3.76**	*1* **2.38**	*19* **2.85**
16	Others	*5* **3.91**	*7* **6.93**	*2* **2.15**	*5* **2.94**	*2* **1.50**	*1* **2.38**	*22* **3.29**
	Total	*128* **100**	*101* **100**	*93* **100**	*170* **100**	*133* **100**	*42* **100**	*667* **100**

natural foods promoting consumption of fruits & vegetables, cereals, pulses, millets etc than others among all NUTRE topics. English newspapers, apart from fruits and vegetables covered more percentage of articles on obesity, processed foods like chocolates, ice-creams and beverages (including liqour) among other NUTRE topics.

Various articles on the topic of 'chocolates' appeared in English newspapers found to be typical and could be confusing. For example, a news item appeared in Deccan Chronicle titled "Dark chocolate good for health" another item published in the same newspaper reads the caption "Chocolates bad for bones". It was mentioned that both the articles are based on the results of two different studies, but only the latter story (chocolates bad for bones) consists details of the place and persons carried the research including the name of the renowned journal in which the study paper was published. An article titled *"Soundarya Pizzalu"* (Pizzas for beauty) appeared in Andhra Jyothi reads 'physical exercise and make-up are not required to look beautiful, if one consumes pizzas regularly, he/she can prevent wrinkles on the face'. Research done by a University student and a proprietor of a Hotel (without quoting names and places) was mentioned as source of the information to this article. English newspapers seem more inclined to publish articles on alcoholic drinks. Particularly, Deccan Chronicle and Times of India published more number of articles (12 and 6 respectively) on alcohol in a way that connects with health and nutrition topic. A research finding appeared in Deccan Chronicle titled "Beer after workout is better" is one such news item, which could lead to confusion among the readers. Often, Times of India and Deccan Chronicle published articles on health benefits of wine and red wine and even nutrition tips to get redeem from hangover the 'Day After'. A significant observation of this study was that, Telugu newspapers have not published any article on alcoholic drink connecting with health and nutrition topic.

CONCLUSIONS

Telugu dailies covered more number of articles and visuals on nutrition-related topics as compared to English newspapers. The grading scores indicated that, English dailies has gave more prominent space to NUTRE articles than Telugu language newspapers. Telugu dailies published more percentage of articles on natural foods, fruits, vegetables, cereals, pulses, millets, etc than other nutrition-related topics. English newspapers covered more percentage of articles on obesity and processed foods (chocolates, ice-creams, beverages) than others among all NUTRE topics.

Of the three English newspapers studied, over-emphasis of a few research findings was observed on two of them. Similar trait was observed in one of the Telugu newspaper. Though, appearance of such kind of information is rare, it cannot be ruled out as insignificant. Since, media have potential influence on the minds of readers, information appears in daily newspapers is expected to be authentic, particularly in relation to health and nutrition news. In order to avoid even slightest detraction in publishing nutrition related topics in print media, there is a need for synergetic efforts between Journalists covering health/nutrition topics and scientists/ experts in the field of diet and nutrition.

V. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

1 MICROBIOLOGICAL RISK ASSESSMENT OF STREET FOODS WITH SPECIAL REFERENCE TO POULTRY PRODUCTS

Microbiological risk assessment (MRA) is a scientific tool that can be used to evaluate the level of exposure and the subsequent risk to human health due to a specific organism in one or more foods *(Cassin et al., 1998*). The overall objective of risk assessment is to provide estimates on the probability of disease occurrence using a well structured approach based on four steps hazard identification, hazard characterization (dose response), exposure assessment and risk characterization *(Syposs et al., 2004)*.

In the context of rapid urbanization, street foods are becoming increasingly important as both a cheap and quick meal option as well as an income generating strategy. Many countries do not regulate street vendors and it is therefore possible to enter street food vending with a relatively low start up cost, making this activity attractive to many low income urban residents.

Quality and safety are two common concerns cited with regards to street foods (Kennedy *et al*., 2004). If the street foods are prepared from high risk foods like poultry, chicken, the concern is much more.

OBJECTIVES

1. To analyze various poultry products sold by street vendors in Hyderabad for microbial contamination.
2. To isolate and identify the bacteria in them.
3. To carry out the intake of poultry products using a questionnaire among consumers and assess the risk.

METHODOLOGY

The most common recipes were identified as Chicken Fried Rice, Chicken Noodles, Chicken Manchuria, Chilli Chicken and Ginger Chicken. A process flow diagram was developed to identify the Critical Control Points(CCP) in high risk foods. Proportionate stratified random sampling was adopted for sample collection. A total of 376 samples including chicken fried rice, chicken noodles, boiled noodles and boiled rice from circle 1, 2, 3, 4, 5, 6 and 7 of Hyderabad were collected and analyzed for microbiological contamination.

Direct microscopic examination of the samples and culture were carried out to identify the bacteria and to enumerate their number. 20g of the food sample was weighed and added into 200 ml of sterilized buffered peptone water. After thorough mixing of the sample 0.1 ml of the sample was inoculated on selective media like XLD (Xylose Lysine Deoxycholate Agar) for *Salmonella*, MSA (Mannitol Salt Agar) for *S.aureus and* BCA (Bacillus Cereus Agar) for *B.cereus*

After incubation period at 37°C for 24 hrs the colonies were observed. The identification of pure culture from food samples was done by studying colony characteristics, microscopy, and motility test and biochemical characteristics.

The statistical analysis was done by descriptive analysis (mean, standard deviation, Standard Error, Minimum, and Maximum) for each category of the groups. Difference between the group were tested by

non parametric Kruskal-Wallis ANOVA considering the heterogenicity of variance and individual pair difference was done by Mann-Whitney U Test (SPSS 14.8 windows version was used).

RESULTS

1. Chicken fried rice and chicken noodles are selected as high risk poultry street foods to carry out risk assessment. Sixty percent of samples are contaminated either with *Bacillus cereus or Staph aureus.* Contrary to general perception these products are not contaminated with *salmonella.* The most prevalent serovars isolated were *Staphylococcus aureus* and *Bacillus cereus.*
2. Statistically significant differences were observed in the levels of contamination in different circles of Hyderabad. *Bacillus cereus* count isolated from chicken fried rice of circle 1 ($P<0.001$) was significantly different from circles 2, 3, 4, 5, 6 and 7. The *Bacillus cereus* count was more in circle 1 (4.6 log cfu/g) when compared with circle 2 (1.90 log cfu/g), 3 (3.47 log cfu/g) and 4 (3.17 log cfu/g).
3. Similarly a significant difference was observed in *Staphylococcus aureus* count isolated from chicken noodles of circle 1 ($P<0.01$), 3 and 7. The *S. aureus* count was more in circle 1 (3.85 log cfu/g) when compared with circle 3 (2.71 log cfu/g) and it was not detected in circle 7.
4. It was found that *Salmonella sps* is present in salads (3.27 log cfu/g) and hand washings of the food handler (3.50 log cfu/g). These findings showed that pathogenic bacterial population is high in circle 1 than circle 2, 3, 4, 5, 6 and 7 and it is indicated that bacterial population varies from locality to locality.
5. Bacterial contamination was very high in salads and drinking water. *Salmonella* contamination was found more in salads than in the street food. It was found that *Salmonella sps* is present in salads (3.27 log cfu/g) and hand washings of the food handler (3.50 log cfu/g).
6. A survey on 217 consumers was done to elicit information on consumption of poultry based street foods. The profile of the consumers who had street foods indicated that 47.2% of the consumers belong to the age group between 16-20.
7. 18% of the consumers had complained that they had symptoms like pain in abdomen, vomiting, diarrhea after consumption of street foods.

Table 1. Microbiological quality of poultry street foods

Food	Total no. samples analysed	No. contaminated (%)	+ve for B.cereus (%)	+ve for S.aureus (%)	+ve for Salmonella (%)
Chicken fried rice	94	53(56.3)	49(52.1)	22(23.4)	0(0)
Chicken noodles	94	60(63.8)	56(59.5)	23(24.5)	1(1.0)
Boiled rice	94	57(60.6)	48(51.0)	3(3.2)	6(6.4)
Boiled noodles	94	72(76.5)	62(66.0)	40(42.6)	8(8.5)

Table 2. Microbiological quality of salads, drinking water and hand washings

Samples	No. of samples Analysed	Samples positive for *B.cereus (%)*	Samples positive for *S.aureus (%)*	Samples positive for *Salmonella (%)*
Salads	62	25(40.3)	23(37.0)	14(22.5)
Drinking water	56	16(28.5)	7(12.5)	5 (8.9)
Hand washings	28	19(67.8)	14(60.7)	6 (28.5)

8. Lactic acid can be used as preservative to control the foodborne pathogens in poultry chicken. So the effect of Lactic Acid on the growth of selective foodborne pathogens (*Salmonella sp, Staphylo-coccus aureus* and *B.cereus*) was studied *in vitro* and *in vivo* experiments. One percent Lactic acid was effective against all the bacterial pathogens at 1 min contact time (*in vitro*), 1% LA was effective against mixed bacterial culture at 1 min. contact time (*in vitro*), The Effect of 3% Lactic Acid on the growth of selective foodborne pathogens in raw chicken by piercing method was very effective (up to not detectable level) (*in vivo*).

9. The exposure assessment indicated that the mean microbial load of *B.cereus* in chicken fried rice is 3.0×10^4 cfu/g, and The mean exposure per serving was, 1.3×10^7 cfu/g. The mean exposure of *S.aureus* per serving was 1.7×10^6 cfu/g. The mean exposure of *B.cereus* in Chicken Noodles per serving was 3.7×10^6 cfu/g. The mean exposure of *S.aureus* in Chicken Noodles per serving was 3.9×10^5 cfu/g.

10. Conventional risk assessment is based on the infective dose of each pathogen per gram of the incriminated sample found in epidemiological studies. Conventional risk assessment indicated that only 2% and 4% of the samples of CFR and CN had infective doses of pathogens respectively. If MRA is done as suggested by Codex, the risk increases to 32 and 35% of the samples of CFR and CN respectively.

11. Preliminary studies to identify the source of salmonella contamination in green salads indicate that the fresh vegetables analysed from the market are free from the salmonella contamination. Food handlers hand washings were contaminated, while knives and wooden plank used for the cutting the vegetables were free from contamination. From these results it may be presumed that salads are contaminated by unhygienic handling by food handlers.

CONCLUSION

1. *Bacillus cereus* and *Staph. aureus are* major pathogens found in poultry street foods.

2. *Salmonella* contamination of the Salads is due to improper handling by food handlers.

3. There could be an under estimate of risk of food borne disease if conventional method is used.

4. There is a need to carry out MRA based on codex guidelines to precisely estimate the risk.

5. Risk evaluation and identifying risk factors would help in curtailing foodborne infections occurring due to consumption of street foods. To achieve this awareness must be created among vendors to ensure hygiene and quality of food.

2 IN VITRO CHELATING POTENTIAL OF THIAMINE WITH LEAD

Lead toxicity remains a public health problem among all categories of population due to chronic low level lead exposure. The use of chelating agents viz.Calcium ethylenediaminetetraacetic acid (CaEDTA), for lead detoxification has limitation as they are potential toxic. The use of Dimercapto succinic acid (DMSA) in mobilizing lead from the bone and other tissues due to lipophobic nature is in controversy.

Since the past one decade, the beneficial role of vitamin B and Vitamin C in reducing the effects of lead toxicity and mobilizing it from body as a safe effective agent is being considered. Our previous *in vivo* studies on rats have suggested potential preventive / therapeutic effect of thiamine (AR-1991). The NMR spectroscopy studies using *in vitro* technique demonstrated that thiamine has weak chelating potential to form complexes with lead. However the concentrations required for thiamine to chelate with lead is not assessed. In the present investigation the chelating potential of thiamine using UV-Visible Spectroscopy, Fluorescence and determination of lead by GF-AAS was measured.

HYPOTHESIS

Thiamine can chelate lead under *in vitro* condition.

OBJECTIVES

To assess *in vitro* chelating potential of thiamine with lead.

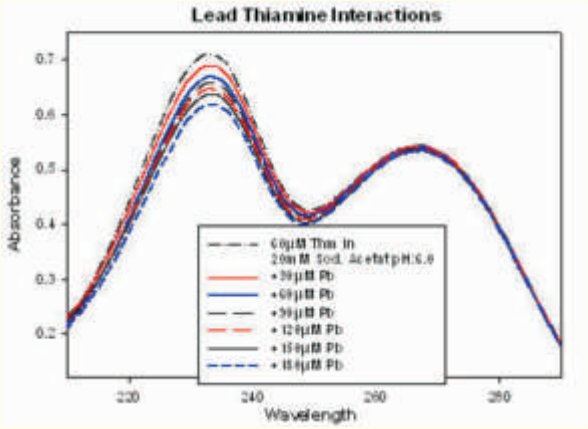
METHODOLOGY

In *In vitro*, the chelating potential of thiamine with lead was determined by (i) UV-Visible spectrophotometry, (ii) Fluorimetry and (iii) solubulization property of lead in presence and absence of Thiamine at physiological pH using Graphite Furnace –AAS.

RESULTS

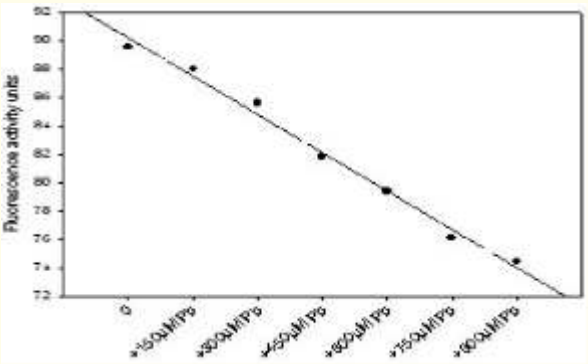
The characteristic absorption maxims (λ max: 231nm and 270nm) for thiamine HCl (60µM) in Sod. Acetate buffer (20mM, pH 6.0) were determined by UV-visible spectrophotometer. A decrease in λ max at 231nm was recorded only with increasing concentration of Lead acetate of 30µM to 180µM. (Fig. 1).

Fig. 1 Change in Characteristic Absorption Maxims of Thiamine with increasing conc. of Lead



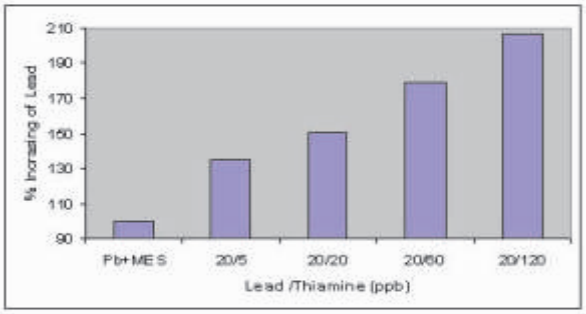
The decrease in Fluorescence activity of Thiachrome, oxidized product of thiamine, was observed with increasing concentration of Lead at 150µM to 900µM on fluorescence spectroohotometer. (Fig.2)

Fig. 2. Change in Fluorescence activity of Thiamine with increasing conc. of Lead



The solubility of lead at physiological pH in an inert buffer was quantified in absence and presence of thiamine on GF-AAS. The increase in solubility of lead with increasing concentration of thiamine at physiological pH was observed after determining the Pb levels on GF-AAS. (Fig.3)

Fig. 3. Change in solubility of Lead in Presence and absence of Thiamin



CONCLUSIONS

The study results using various techniques demonstrated that thiamine has therapeutic potential of chelation with lead at low concentration.

In view of this thiamine can be used for treatment of low lead level toxicity.

VI. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

SERVICE ACTIVITIES

1 Breeding and Supply of Animals:

During the 9 months period 28,396 animals were bred and out of which 25,299 animals were supplied for research including the parent institution. There was a decrease of 2.6% in breeding and 3.0% in the supply of animals. The income generated from animal supply was Rs.32.68 lakhs. The details of individual animals species and strains bred and supplied are shown in Table 1 and 2.

2 Supply of animal Feed

Stock animal feed

A Total of 22,871 kg of animal feed (21,521 kg of rat/mouse feed; 1350 kg of g.pig/ rabbit feed) was supplied during the period generating an amount of Rs.21.27 lakhs. There was increase of 2.3% in the supply of feed and 27.4% in the amount generated during the reported period.

Experimental Animal Feed

Need based supply of experimental animal feed of 350 kg, was continued during the period and the details are shown in Table 3. (Including two of our institutional experimental feeds)

3 Supply of Tissues/Organs /Blood & Blood Products:

During this period a total of 1621ml of blood & blood products(blood-25 ml, sera-141 ml, plasma-1335 ml) were supplied to 7 different institutions on 28 occasions. and a sum of Rs.2, 50,066 was realised. Apart from above,120 ml of blood was also supplied to the institute.

In addition during this period organs from mice and rats were supplied to 2 institutions on 3 occasions and a sum of Rs 22,660/- was realized.

4 Health Monitoring

A total no of 429 samples from mouse and rat colonies were screened for microbiological monitoring (bacterial) during the period. The samples were taken from 126 Mice [BALB/c 28; C57/6J 06; NCI 22; FVB/N 18; Nude hetero 22; Swiss 30] and 160 rats [WNIN-46; S.D-84; Fischer 10; Kyoto 06; CFY 08; Holtzman 06]. In addition, fecal samples were also taken for monitoring from 20 hamsters , 45 guinea pigs and 45 rabbits . The other samples included, 09 from water; 09 from feed, 09 from bedding and 06 from others like water bottles and canopies.

The results of the bacterial screening is given in table 1 and 2. In both the mouse and rat strains organisms like Klebsiella ,Corynebacterium, Proteus, Staphylococus , Streptococcus, Listeria monocytogenes were found. In general, the data showed that more animals are affected with advancement in age.

Additionally, mice (84 no) and rat (250 no) strain sera samples were screened for viruses. The rat sera was also tested for bacteria like leptospira, *clostridium piliformis* (CPIL), and cilia associated respiratory bacilli (CARB)]. The results of the screening are given in Table 3 and 4 for mice and rats respectively. The most prevalent virus among the all the mouse strains was MPV.

While swiss mouse was found to have only the virus MPV, all the mouse strains were found to be free from EDIM. 28 out of 46 rat samples tested positive for mycoplasma while CPIL was found in 1 sample and CARB was found in 7 samples of the rat strains screened.

Table 1. Details of breeding and supply of different species and strains of laboratory animals during the period from 1.4.08 to 31.12.08

Sl. No	Species	Strain or Breed	Stock As on	Total Number of animals							Balance as on
			1.4.08	Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp. Old/Sick age	31.12.08
1	Mouse	BALB/c An. N (inbred)	432	4404	4836	486	3585	4071	-	-	765
		C57BL/6J (inbred)	996	3748	4744	22	3979	4001	12	126	605
		N:NIH(S) Nude (inbred)	152	570	722	187	160	347	256	-	119
		NCr.Nude	342	844	1186	357	310	667	286	-	233
		FVB/N (in bred)	308	67	375	279	-	279	15	-	81
		Swiss (in bred)	785	4281	5066	740	3650	4390	109	-	567
2	G. Pig	N:HART (Hartley)	266	615	881	24	564	588	60	-	233
		N:NIH (Coloured)	132	345	477	24	197	221	27	-	229
3	Rabbit	New Zealand white	106	75	181	43	87	130	13	-	38
4	Monkey	Macaca mulatta (Rhesus)	24	-	24	-	-	-	-	-	24
	TOTAL		**3543**	**14949**	**35890**	**2162**	**12532**	**14694**	**778**	**126**	**2894**

Table 2. Details of breeding and supply of different species and strains of laboratory animals during the period from 1.4.08 to 31.12.08

Sl. No	Species	Strain or Breed	Stock as on	Total Number of animals							Balance as on
			1.4.08	Bred during the period	Available	Supplied to NIN	Supplied to other Instts.	Supplied Total	Died	Disp. Old Age/ Sick	31.12.08
1	Rat	CFY/NIN (inbred)	94	58	152	-	-	-	42	18	92
		Fischer 344 N (inbred)	100	144	244	-	20	20	92	-	132
		Holtzman (inbred)	53	18	71	-	-	-	10	27	34
		SD (Sprague Dawley) (Outbred)	749	3591	4340	721	2265	2886	260	-	1094
		Wkyoto (inbred)	68	20	88	-	-	-	26	20	42
		WNIN (inbred)	1208	7635	8843	49	6234	6683	130	735	1295
		WNIN/GR-Ob	628	577	1205	137	-	137	52	245	671
		WNIN/Ob-Ob (inbred)	782	351	1133	199	-	199	68	200	666
2	Hamster	Golden (inbred)	268	1053	1321	-	580	580	24	230	487
3	Sheep		1	-	1	-	-	-	-	-	1
Total			3951	13447		1506	9099	10605	704	1475	4514

Percentage of animals supplied to other Institutions: 76%

Table 3. Experimental Feed

No.	To whom supplied	Type of diet	Qty (kgs)
1	NIN	NTP 2000 diet	80
2	NIN	Low protein diet	80
3	NIN	DAG Oil diet	9
4	CCMB	Cholesterol diet	5
5	NBRC	Iron deficiency diet	20
6	NBRC	High protein diet	20
7	NBRC	Low protein diet	20
8	SK University	Fructose diet	70
9	Hamdard University	High Fat diet	15
10	MRDC	Fructose diet	15
11	CCMB	Maltodextrine diet	16
TOTAL			350

Table 4. Pathogenic microorganisms isolated from different strains of mice

Organisms Isolated	BALB/c (28)		C-57/6J (6)		Swiss (30)		FVB-N (18)		Nude-Hetero(22)		Nude NCI (22)	
	2m (4)	>6m (24)	2m (2)	>6m (4)	2m (6)	>6m (24)	2m (4)	>6m (14)	2m (6)	>6m (16)	2m (6)	>6m (16)
Actinobacillus spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bordetella bronchiserica	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CAR bacillus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Corynebacterium kutscheri	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Klesbsiella spp	ND	ND	ND	**01**	ND	ND	ND	**04**	ND	**04**	ND	**04**
Pasterurella spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pseudomonas aeruginosa	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Proteus	ND	ND	ND	ND	ND	**05**	ND	ND	ND	ND	ND	ND
Salmonella spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Staphylococcus aureus	**03**	**12**	**01**	**02**	**04**	**24**	**02**	**12**	**2**	**16**	**02**	**16**
Streptobacillus moniliformis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Streptococcus pneumoniae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Streptococcus spp	**03**	**14**	**01**	**02**	**04**	**24**	**02**	**12**	**3**	**16**	**02**	**16**
Yersinia pseudotuberculosis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: NOT DETECTED

Table 5. Pathogenic micro organisms isolated from different strains of rats

Pathogenic organisms to be monitored	WNIN (46)			S D (84)		Fisher (10)		Kyoto (06)		CFY (08)		Holtzman (06)		Hamster (20)
	2m (18)	4m (8)	>6m (20)	4m (12)	>6m (72)	4m (4)	>6m (6)	2m (2)	4m (4)	4m (4)	>6m (4)	2m (4)	4m (2)	>6m (20)
Actinobacillus spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	--
Bordetella bronchiserica	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	--
CAR bacillus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	--
Corynebacterium kutscheri	ND	**04**	**20**	**02**	**06**	**01**	**02**	**01**	**03**	**01**	**03**	ND	**01**	**--**
Klesbsiella spp	ND	**01**	**15**	**06**	**05**	**02**	**04**	**01**	**01**	**01**	**01**	ND	**01**	**04**
Pasterurella spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**--**
Pseudomonas aeruginosa	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**--**
Proteus	ND	ND	**05**	**04**	**04**	ND	**04**	ND	**01**	ND	**01**	ND	ND	ND
Salmonella spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**--**
Staphylococcus aureus	**08**	**02**	**08**	**02**	**15**	**02**	**04**	**02**	**03**	**02**	**03**	**03**	**01**	ND
Streptobacillus moniliformis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**--**
Streptococcus pneumoniae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**--**
Streptococcus spp	**08**	**02**	**08**	**02**	**10**	**02**	**06**	**02**	**02**	**02**	**02**	**02**	**01**	ND
Yersinia pseudotuberculosis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Giardia spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**12**

ND: NOT DETECTED

Table 6. Results of mice sera samples tested

S.No	Virus	Total samples Tested	Mice strains				Result
			Swiss	C57	BALB/c	FVB	
1	GDVII	14	0/2	4/4	4/4	4/4	12/14 positives
2	Hantaan	14	0/2	0/4	1/4	0/4	1/14 positives
3	Ectro	14	0/2	0/4	1/4	0/4	1/14 positives
4	EDIM	14	0/2	0/4	0/4	0/4	All negatives
5	MHV	14	0/2	4/4	2/4	2/4	8/14 positives
6	MPV	14	2/2	1/4	4/4	3/4	10/14 positives
Total		84					

Table 7. Results of rat sera samples tested

No	Virus / organism	Total samples Tested	Rat strains						Result
			WNIN	SD	Fisher	Holt	CFY	Kyoto	
1	Sendai	46	0/8	0/14	0/6	0/6	0/6	0/6	All negatives
2	Mycoplasma	46	7/8	8/14	1/6	3/6	6/6	3/6	28 positives
3	GDVII	12	0/2	0/2	0/2	0/2	0/2	0/2	All negatives
4	Hantaan	12	0/2	0/2	0/2	0/2	0/2	0/2	All negatives
5	H1	12	0/2	0/2	0/2	0/2	0/2	0/2	All negatives
6	RPV	12	0/2	0/2	0/2	0/2	0/2	0/2	All negatives
7	CARB	12	0/2	0/2	2/2	2/2	1/2	2/2	7/12 positives
8	CPIL	12	0/2	0/2	0/2	1/2	0/2	0/2	1/12 positives
9	Leptospira Ig M	40	0/5	0/15	0/5	0/5	0/5	0/5	All negatives
10	Leptospira Ig G	46	0/8	0/14	0/6	0/6	0/6	0/6	All negatives
Total		250							

Table 8. Organ abnormality in sick / culling / old age group animals

Species/ Strain	No	Skin	Tumors	Tail	Lungs	Liver	Spleen	Kidneys
Mice								
BALB/c	410	82	4	20	4	3	8	--
Swiss	255	54	--	--	2	4	10	--
FVB/N	335	74	--	--	6	--	--	--
NIH Hetero	110	45	--	--	--	--	2	--
NCI nude	115	52	--	--	--	2	1	--
Total	1225	307	4	20	12	9	21	0
Rats								
WNIN	202	50	4	6	10	10	9	--
SD	259	60	4	--	12	10	8	2
Fischer	10	--	--	--	--	--	4	--
Holtzman	6	--	--	--	--	2	1	--
CFY	6	--	--	--	--	2	--	--
Total	483	110	8	6	22	24	22	2

All rat strains were found to be negative for leptospira and the 5 viruses (Sendai, GDVII, Hantaan, HI, RPV) screened.

Apart from bacteria ecto parasites were found in almost all strains of mice. Endoparasites like Taenia taeniformis *Syphacia obvelata* were found in WNIN, SD, Fischer and Kyoto rats. Giardia was the only pathogenic endoparasite isolated from hamster.

As part of health monitoring activity 30 rabbits supplied to Pre Clinical Toxicology (PCT) were examined for various pathogenic organisms and parasites during their quarantine period.

Details of organ abnormalities observed in sick and old age group animals (mice and rats) while conducting necropsy are given in Table 5. Predominantly abnormalities like skin lesions, roughened tails, liver cysts, renal tumors, lung emphysema and splenomegaly were observed in these animals.

Human Resource Development:

? In the Junior level course, LATTC, 9 participants were trained. The senior level course, LASTC was not conducted due to inadequate number of applications.

? Ad hoc training was given to one WHO sponsored candidate for a period of 3 weeks.

? The center also took 5 students of B.Tech Biotechnology and M.Sc., for their dissertation work in animal physiology and bacteriology.

Research Support

Pre Clinical Toxicology (PCT)

During the period 8 research projects from PCT was approved by IAEC for implementation. Out of these two studies are under progress, and other studies are yet to be initiated.

VII. PRE-CLINICAL TOXICOLOGICAL STUDIES

1 SAFETY EVALUATION OF AB-FN-02 HAVING POTENTIAL ANTI-OSTEOARTHRITIC ACTIVITY

The product AB-FN-02 is an improved formulation of AB-FN-01, which is reported to have anti-inflammatory activity, and recommended for clinical use in patients with osteoarthritis .The combination contains two plant products (*Semicarpus anacardium* and *Tinospora cardifolia*). The experimental evaluation in various inflammatory models has shown potential therapeutic benefits. Since *Semicarpus anacardium* is reported to have few adverse effects, its safety evaluation as per the regulatory guidelines is one of the important aspect .The present investigation is being planned to undertake the Pre Clinical Toxicological profile of the AB-FN-02 as per WHO guidelines and schedule 'Y' of Drug and Cosmetic Act 1940.

METHODOLOGY

The present investigation involves Acute toxicity test (14 days) in Swiss albino mice and Sprague Dawley rats and Long Term toxicity test (120 days) in Sprague Dawley rats. In acute toxicity test, mice and rats were exposed once to highest dose (10 times of intended therapeutic dose) by oral gavage and observed for lethality. Long term toxicity test have been conducted in Sprague Dawley rats, which were equally divided into five groups viz., Vehicle control (VC), Therapeutic dose (TD), Traditional Therapeutic dose(TTD) group, Average dose (AD, five times of TD) and High Dose (HD, ten times of TD). The TTD group is specially introduced in the study so as to mimic the clinical treatment schedule where the drug is administered along with milk. The dosage schedule in long-term toxicity test includes oral administration of the test compound in varied doses daily for 120 days. The rats were monitored bi-weekly for live phase, cage side, physical and neurological parameters. In addition, the blood and serum samples of animals collected at various time points have been evaluated for clinical chemistry and hematology profile. All major organs were collected for gross necropsy and histopathological evaluation after euthanization. Genotoxicity studies were also carried out.

RESULTS

There were no pre-terminal deaths of mice and rats in acute toxicity test till 14th day after single administration of 10 times of Therapeutic dose. Pre-terminal deaths in long-term toxicity test were observed. In TD - 8.3%, AD - 41.6% & HD - 58.3% deaths were recorded. No lethality was observed in Control and TTD group.

In rats that survived, no difference in body weight gains, live phase, physical activity and neurological activity was observed between the control and test groups throughout the study period. No major abnormal clinical signs were observed in any group of animals at the time of test compound administration. The biochemical and hematological profile was in normal range in all groups of surviving animals. Incidental lesions in pancreas of VC group lymphoid tissue in the sub mucosa and lymphoid hyperplasia in various groups of animals were recorded. However it may not be attributed due to exposure to the test material. The micro nucleated PCES were significantly high in AD and HD group, suggesting cytotoxicity at higher dose level.

CONCLUSION

There was no lethality on single exposure (Acute study) of ten times of the intended therapeutic dose in mice and rats. In Long

term toxicity study pre-terminal deaths were observed which were dose related. Safety profile of ABFN formulation was recorded in rats exposed to test compound as per traditional treatment. The mortality was dose dependent with maximum lethality of 50% in High dose group. In the animals that survived, apart from routine activity, the biochemical and haemetological profile was found to be normal. No treatment related histopathlogical alteration in all organs investigated. The test compound has potential cytotoxicity at higher dose.

2 PRE-CLINICAL TOXICITY EVALUATION OF SHEA OLEIN

Shea Olein is an oil fraction derived from shea butter obtained from nut of the tree *Butyrospermum parkii*. It is composed of triglycerides containing Oleic acid and two saturated fatty acids. The Shea nut oil after fractionation yields shea stereane and shea olien. The solid fraction, shea stearene is mainly used in the manufacture of coca butter equivalents used in the manufacture of chocolates. The liquid fraction namely shea olien is used in Europe and other countries for the manufacture of bakery shortenings and margarine. In few African and European countries it is used as a source of edible oil.

In India also the demand for edible oil and requirements of such products in bakery industry is growing. Ms. Foods, Fats & Fertilizers Limited, a large manufacturer of edible oils, vanaspathi, bakery shortening and margarines, is keen to promote the shea stearine for manufacture of coca butter equivalents and the shea olien for the manufacture of Vanaspathi, bakery shortening and margarine with 20% blend of Shea Olein in Vanaspathi. In order to promote such products for human consumption, the pre-clinical safety is undertaken in Mice and Rats as per the regulatory requirement of PFA.

METHODOLOGY

The present investigation involves Acute toxicity test (14 days) in Swiss albino mice & SD rats; and Sub-chronic toxicity test (28days) in SD Rats.

Acute toxicity test has been conducted in 20 Mice (10M + 10F) and 10 Rats (5M + 5F) aged between 6-7 weeks. The weight range of the Mice was 18-25gm and Rats between 140-160gm. All the animals were exposed once to 10X of Recommended Dietary Intake of test compound through oral gavage to observe activity and lethality.

Sub-Chronic toxicity test has been conducted in 72 SD Rats (36M + 36F) aged 4-6 weeks, weighing 110-150gms which were divided equally in to six groups. Group I served as groundnut oil control, Group II as Vanaspathi - vehicle control and received Ground nut oil and Vanaspathi in diet respectively. The Group III, IV and V referred as test groups received 20%, 30% and 50% Shea Olein in Vanaspathi through diet respectively. In addition Group VI served as Poor Man's Diet group which received 20% of *Shea Olein – Vanaspathi* through diet which was deficient in proteins & calories by 30%. All the animals were provided with powder diet containing test material after conditioning for 10 Days.

The animals in all groups were monitored bi-weekly for live phase, cage side, physical, neurological and allergenicity parameters. The blood samples of animals were collected at two time points viz., 48hrs, and 15days after giving the test diet, for last time to evaluate hematology and clinical chemistry profile. Similarly 50% of animals were euthanized at 48 hours and remaining on 15th day after last exposure to test material to collect major organs. The gross necropsy of all organs was conducted and subjected to Histopathology

examination. The Genotoxicity profiles have also been evaluated by Rodent Bone marrow micro nucleus assay.

RESULTS

In the acute toxicity study, one male mouse died on 6th day (5%) while there was no mortality in rats fed Shea Olein at 10 times of RDI once orally by gavage till 14th day.

In Sub-Chronic toxicity test, no mortality was recorded till end of the experiment. The body weight gains, live phase, physical activity and neurological observations were not different between the animals fed regular diet, diet containing Vanaspathi and 20%, 30% and 50% Shea Olein in Vanaspathi and protein/calorie deficient diet till 28days. Even the biochemical and hematological profiles in all groups on 48hrs and 15days of last test diet exposure were in normal range. At necropsy, no gross lesions were observed in all organs collected in any group of animals. No significant differences were observed in the histopathology of various organs between control, vehicle and test groups.

CONCLUSION

In Acute toxicity test mice and rats fed orally once with Shea Olein at 10 times of RDI did not show any abnormal activity and lethality, except for pre-terminal death of one male mouse on sixth day.

In the Sub-Chronic test, rats fed 20%, 30% & 50% of Shea Olein in Vanaspathi with regular powder diet and 20% Shea Olein - Vanaspathi in protein/ calorie deficient diet, the live phase of activity, gain in body weight, food intake physiological activity and neurological activity were normal and similar to animals fed standard diet and Vanaspathi alone.

There was no abnormality in hematological and biochemical profiles in any groups of rats. No gross abnormality was observed at necropsy. No evidence of histopathology changes due to test material were recorded. No genotoxicity was also recorded. The Shea Olein at 20% - 50% in Vanaspathi under experimental condition has been found to be safe.

3 PRE-CLINICAL TOXICITY EVALUATION OF DAG OIL

Extensive research over the past 40 years has clearly established the role of dietary fat in health and disease conditions. Based on this various health agencies and professionals have made recommendations to cut down the over all fat intake and consume good quality fat with optimal levels of saturated, mono unsaturated and polyunsaturated fatty acids. Of late, the emphasis has been on the development of the edible oils with altered characteristics that influence the fat metabolism and affect body weight. Such products are diacylglycerol (DAG)-rich oils and medium-chain triacylglycerol (MCT)- rich oils. IICT has successfully adapted the Japanese technology for the production of DAG-oil within India. However efficacy and safety evaluation for promoting health benefits is a regulatory requirement.

METHODOLOGY

The present investigation involves acute toxicity test (14 days) in SD rats; and Sub-chronic toxicity test (90 days with additional 15 days as recovery phase) in SD Rats.

Acute toxicity test has been conducted in 10 rats (5M + 5F) aged between 4-6 weeks. The weight range of the rats was 80-110g. All the animals were exposed once to 10X of Recommended Dietary Intake of test compound through oral gavage to observe activity and lethality.

Sub-Chronic toxicity test has been conducted in 36 SD rats (18M+18F) aged 4-6 weeks, weighing 110-150gms which were divided equally in to six groups. Group I served as 20% DAG, Group II 10% DAG,

Group III 10% TAG. All the animals were provided with powder diet containing test material after conditioning for 20 Days .

The animals in all groups were monitored bi-weekly for live phase, cage side, physical, neurological and allergenicity parameters. The blood samples of animals were collected at two time points viz., 48hrs, and 15days after giving the test diet, for last time to evaluate of hematology and clinical chemistry profile. Similarly 50% of animals were euthanized at 48 hours and remaining on 15th day after last exposure to test material to collect major organs. The gross necropsy of all organs was conducted and subjected to Histopathology examination. The Genotoxicity profiles have also been evaluated by Rodent Bone marrow Micro nucleus assay.

RESULTS

In the acute toxicity study, no mortality in rats fed DAG Oil at 10 times of RDI once orally by gavage till 14th day.

In Sub-Chronic toxicity test, one female rat died on 85th day (3%). The body weight gains, live phase, physical activity and neurological observations were not different between the animals fed regular diet, diet containing DAG Oil 20%, 10% and 10% TAG Oil till 90days. Even the biochemical and hematological profiles in all groups on 48hrs and 15days of last test diet exposure were in normal range. At necropsy, no gross lesions were observed in all organs collected in any group of animals. No significant differences were observed in the histopathology of various organs between vehicle and test groups.

CONCLUSION

In Acute toxicity test rats fed orally once with DAG Oil at 10 times of RDI did not show any abnormal activity and lethality.

In the Sub-Chronic test, rats fed with 20%, 10% DAG Oil & 10% of TAG Oil with regular powder diet, no pre terminal death except one female rat on eighty fifth day. the live phase of activity, gain in body weight, food intake physiological activity and neurological activity were normal and similar to animals fed experimental control TAG Oil alone.

There was no abnormality in hematological and biochemical profiles in any groups of rats. No gross abnormality was observed at necropsy. No evidence of histopathology changes due to test material were recorded. No genotoxicity was also recorded. The DAG Oil at 20% - 10% under experimental condition has been found to be safe.

VIII.OTHERS (TECHNICAL/CASE STUDIES)

1 WOUND HEALING IN STREPTOZOTOCIN INDUCED DIABETIC SPRAGUE DAWLEY RATS (IICT)

Wound healing is a complex and highly integrated cascade of events involving four distinct but overlapping phases: hemostasis, inflammation, proliferation and remodeling. At site of injury, platelets get exposed to collagen to release various clotting factors, platelet derived growth factors (PDGF), transforming growth factor beta (TGF-β) etc. In the inflammatory phase, stimulated neutrophils release proteases and reactive oxygen species. Microphages expedite the phagocytic removal of foreign materials & damaged tissues and facilitate release of more PDGF and TGF-β.

Fibroblasts migrate into the wound area and produce the collagen needed for wound repair. The epithelial cells migrate from free edges. Replacement of the granulation tissue with collagen fibres takes place followed by devascularization of the granulation tissue which eventually leads to the formation of a scar over the wound area. Orderly events are lost in cases of impaired or chronic wounds, such as diabetic wounds.

Excessive infiltration by neutrophils with their associated reactive oxygen species (ROS) and degradative enzymes locks the chronic ulcers into a state of lasting inflammation. The neutrophils release the proteolytic enzyme elastase capable of destroying various healing factors including PDGF-B and TGF-β. Among the multitude of factors contributing to the pathogenesis of chronic wounds, deficiency of growth factors is of paramount importance.

Exogenous application of PDGF-B in chronic non-healing wounds requires large and repeated doses. Short shelf life and inefficient delivery to target cells are two additional concerns associated with direct topical administration of growth factors.

This is circumvented by gene therapy approach in which genes (encoding various growth factors) along with gene carriers are administered, for inducing expression of therapeutic protein, such as PDGF-B. Modes for delivering the therapeutic genes include administration of naked DNA, viral transfection, liposomal delivery etc. Because of their least immunogenic nature, robust manufacture, ability to deliver large pieces of DNA and ease of handling & preparation techniques, liposomes are used as non-viral transfection vectors for use in gene therapy.

Lipopeptide with a RGDK tetrapeptide sequence (RGDKlipopeptide 1) selectively target genes to α5β1 integrin receptor and tumor vasculature. Fibroblast also expresses α5β1 integrin receptor on its cell surface, so RGDK-lipopeptide 1 should deliver growth factor encoded genes to wound beds (presumably via the α5β1integrin receptors of fibroblast cells) in non-viral gene therapy of chronic wounds.

HYPOTHESIS

A single administration of rhPDGF-B plasmid, the RGDK-lipopeptide 1 and cholesterol (as auxiliary lipid) is capable of healing wounds in streptozotocin-induced diabetic Sprague-Dawley rats (as model of chronic wounds).

OBJECTIVE

To evaluate the potential of the RGDK - lipopeptide in healing wounds in diabetic rats.

Animal groupings

No	Drug	Dose	Route	Vehicle	n
1	No treatment	---	---	---	2
2	5% Aq. Glucose	Once	Subcutaneously	5% glucose	2
3	Naked plasmid DNA encoding PDGF-B protein	Once	Subcutaneously	5% glucose	2
4	Lipid-DNA Complexes made with RGDK- lipopeptide and plasmid DNA encoding PDGF-B protein	Once	Subcutaneously	5% glucose	3

MATERIALS AND METHODS

Diabetic Sprague-Dawley (S/D) Rats wound model

SD rats were made diabetic by a single injection of streptozotocin (STZ - 4.5 mg/kg, i.p.) after overnight fasting. Diabetic status was defined as blood glucose levels (non-fasting) higher than 300 mg/dl. Fourteen days after STZ treatment; the backs of the diabetic rats were shaved and the rats were anesthetized with ether solution. A 2.0-cm circular dorsal skin incision was produced with a scalpel down to the level of the loose subcutaneous tissues. For the first 48 h post-wounding, the animals were left undisturbed.

Measurement of wound areas was taken on every alternative day. Wound healing process was monitored by calculating the percent reduction in the wound area calculated with reference to wound area on day zero.

All the animals were sacrificed on the 11th day and the skin samples belonging to various groups mentioned, were studied after staining with H&E as well as Masson's Trichrome stain and evaluated based on following criteria:

PARAMETERS STUDIED

Scab formation

Epithelialization – Complete / Partial – Assessed semiquantitively as % . Complete – squamous epithelium was formed as a continuous layer under the scab, Partial – 50% or less of wound surface.

Fibrocollagenous tissue – Comprised of spindle cells as well as interlacing collagen fibres. Collagenization presence was also semiquantitively assessed as %.

Depth of healing – It was in most cases superficial to the muscle layer while it was through and through in occasional cases.

Granulation tissue.

RESULTS & INFERENCE

Based on histopathology study of both H&E and Masson Trichrome stained sections, it was observed that wound healing was best when the combination treatment (RGDK + PDGF) was employed as compared to other treatment groups. However, DNA treated group was better compared to the control group .

2 ECONOMIC IMPACT OF A FOODBORNE DISEASE OUTBREAK IN HYDERABAD – A CASE STUDY

Foodborne diseases are one of the most widespread health problems in both developed and developing countries *(WHO 2007).* Foodborne diseases in general have caused a major economic impact not only within the food industry but also within the society *(Kazuo 2002).*In India there is no systematic study on out break of foodborne diseases. National foodborne disease surveillance system needs to be developed in India in order to enable effective detection, control and prevention of foodborne disease outbreaks. Therefore in order to create awareness among policy makers an attempt was made to calculate the economic impact of foodborne disease outbreak that occurred at Government Research Institute, Hyderabad, India during 2008.

METHODOLOGY

More than 60 persons attended a meeting followed by a lunch at 2.00pm in a Government research Institute at, Hyderabad, India. All the affected persons were the employees of the Institute. Both vegetarian and non-vegetarian foods were served during the lunch. The food items served in the lunch were chicken rice, mutton (goat meat) rice, green chilli curry, vegetable biriyani, curd, vegetable salad, rumali roti, plain rice, *dile firdous* (Sweet prepared from bottle gourd and desiccated milk product).

A questionnaire was designed and information on sex, age, type of food eaten and quantity, time of food consumption, type of initial symptom, time of initial symptom, whether the affected person has been admitted to the Hospital or treatment taken at home, duration of the illness, days of work lost while ill and medical costs incurred by the affected person were collected. All the affected persons could be contacted in person and interviewed during the study. The loss of wages or productivity loss was calculated by determining each employee's daily income and multiplying by the days of work lost. The expenses incurred by affected persons included traveling charges, physician charge and cost of medicines, cost of oral dehydration solution and the miscellaneous expenditure incurred by the attendants of the sick persons.

Cost of investigation was calculated by determining laboratory personnel daily income multiplied by the working days and traveling charges to visit the hospital in order to verify the case sheets. The persons affected due to the present food poisoning case were Government employees. Medical costs incurred towards the food poisoning case has to be compensate by the Government. Medical bills claimed by the employees were prepared by the staff of the Institute, so the administrative charges were calculated based on the administrative staff daily income multiplied by the working days.

SPSS 15 version was used for the statistical analysis. To establish the impact of a particular food item in the outbreak cross tabs were deduced and association between consumption of certain food and symptoms was performed. Association was ascertained by using chi-square test and odds ratios, which were computed at 95% confidence interval. Logistic regression analysis was performed to find out which of the food items has contributed to the outbreak.

The cost of hospitalization for the number of persons who were treated including the cost of investigation was divided by number of persons to obtain per patient hospitalization cost. The percent of per patient hospitalization cost was calculated by the following formula

$$\text{\% of per patient hospitalization cost} = \frac{\text{Cost per patient x 100}}{\text{Per capita income}}$$

RESULTS

1. Among the 60 persons who attended the lunch, 37 persons were affected by foodborne disease. Among the 37 affected persons 10 were admitted to nearby hospitals. Most of the affected persons were in the age group of 46-50 (28.3%)
2. The economic cost of the foodborne disease outbreak for 60 persons was Rs.91901/- 3. About 20.6% of the expenditure, Rs.18800/- was borne by the affected individual, which includes loss of wages or productivity loss. The cost of hospitalization including medicines was 45.5% i.e. Rs.41423/-
4. The cost of investigation was 19.75% of the total economic cost i.e. Rs 17960/-. The total cost incurred towards traveling charges by the affected persons was 1.7% i.e. Rs 1600
5. The cost of the expenses incurred by the affected individuals towards oral rehydration solution was 1.6% i.e. Rs.1518/- .The cost incurred towards administrative charges was 10.5% i.e. Rs 9600/-
6. The per patient hospitalization cost was estimated to be Rs. 990/- and the percent of per patient hospitalization cost in terms of per capita income for India during 2008-09 was 3.3%
7. The logistic regression analysis showed that it may be *dile firdous* that would have contributed significantly to the incidence of foodborne illness.

CONCLUSION

There is a need to quantify the economic cost of foodborne illness to understand the economic consequences of the foodborne illnesses besides the morbidity.

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 dissemination services like MEDLINE Searches, Proquest Medical Library Full Text Database of journals and other online retrieval activities using the LAN Network of the Institute. Library continued to participate in exchange of data, journals and information using the URL<http://Groups.yahoo.com/group/ICMR Librarians>.

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 Scientific Journals etc., are being routed through the Library and a data-base of the published papers is also made accessible through on-line services using NIN Website (www.ninindia.org).

MODERNISATION OF LIBRARY AND INFORMATION NETWORK

The following work has been taken up and the equipment is procured for strengthening the services of dissemination of Information to the scientists.

a) ICMR has renewed the subscription to **Proquest Medical Library Full Text Datase** of the journals. During the period total of **2244** Proquest ML Full Text Database Searches were made.

b) Subscription of JCCC@ICMR and J-Gate has been renewed by Indian Council of Medical Research through M/s. Informatics India Pvt. Ltd., Bangalore, JCCC@ICMR covers more than **1124** journals received collectively at 24 Institutions/Centres Consortia of ICMR Libraries. And **J-Gate** is an electronic gateway to global e-journals literature. It presently has massive database of journal literature indexed from more than 17, 991 e-journals with links to full text at publisher sites and provides free access to full-text of 1700+ journals with e-author e-mail address and also one can find the availability of the journal in a local library.

c) NIN Library is also a member of NML – ERMED Consortia for accessing more than **1500** + Journals (850 E-Journals + 650 Print).

d) Online Subscription of **5 Core Journals** such as BMJ, LANCET, NATURE, NEJM, SCIENCE has been renewed by ICMR is also accessable.

e) The following equipments were procured for the library

i) HP PC – 3 Nos.

ii) UPS – 3 Nos.

NEW JOURNALS ADDED

Indian Journals

1. Current Trends in Biotechnology & Pharmacy

2. Journal of Environmental Biology

The following library services were expanded as detailed below:

1. NEW ADDITIONS

Books	. . . .	154
Reports	. . . .	415

Journals (New Subs.)	. . . .	2
Thesis / Dissertations	. . . .	5
CDROMS	. . . .	47
ProQuest CD's	. . . .	5
PC Quest CD's	. . . .	22
General CD's	. . . .	20

2. OTHER ACTIVITIES

Journals Bound	. . . .	1,595
Visitors using the Library	. . . .	2,204
Circulation of Books/Journals etc	. . . .	1,069
MEDLINE Abstracts provided	. . . .	1,000
No. of E-mails sent outside	. . . .	1,006
No. of E-mails received	. . . .	7,619
Photocopying (No.of pages)	. . . .	4,11,738
Number of Annual Reports mailed	. . . .	423
No. of Books/Journals received	. . . .	10
No. of Duplicate Journals sent out	. . . .	150
No. of INTERNET Searches provided	. . . .	176
No. of Reprints sent	. . . .	403
Proquest Full Text Database searches provided	. . . .	2,244

3. TOTAL LIBRARY COLLECTIONS

Books	. . . .	17,065
Journals (Bound Volumes)	. . . .	30,937
Journals subscribed for 2008	. . . .	314
Journals received (Gratis/Exchange)	. . . .	320
Microforms (Microfiche)	. . . .	1,080
Slides	. . . .	280
Reports	. . . .	12,465
Theses & Dissertations	. . . .	372
MEDLINE CDROMS Discs	. . . .	383
Current Contents on Diskettes with abstracts	. . . .	664
Proquest (Full Text E-Journals) on CD ROMS	. . . .	49
Total General CD's	. . . .	42

Ph.D PROGRAMMES

RESEARCH SCHOLARS REGISTERED FOR PH.D

Research Scholar/staff	Title of thesis	Guide
1. Aruna B. (2002)	Biophysical characetrisation of resistin	Dr. Nasreen Z. Ehtesham
2. Haseeb A (2002)	Understanding the mechanism of action of PPAR? as a link molecule between obesity, Type 2 diabetes and CHDs	Dr. Nasreen Z. Ehtesham
3. Kiran Kumar B. (2002)	Genetic typing of WNIN/Ob and WNIN/ GR-Ob strains using microsatellite markers	Dr.Giridharan N.V
4. Megha Saraswat (2003)	Screening of aldose reductase inhibitors and antiglycating agents from dietary sources and assessing their anticataractogenic potential	Dr.Bhanuprakash Reddy G.
5. Mrudula. T (2003)	Characterisation and significance of a novel fatty acid elongase of the eye lens	Dr.Bhanuprakash Reddy G.
6. Prashant A. (2003)	Role of scavenger receptor class B1 (SR-B1) in reticulocyte differentiation, absorption of fat and fat soluble vitamins and female infertility using WNIN/Ob rat model	Dr.Vajreswari A.
7. Md.Naseeruddin (2004)	Understanding the role of resistin in inflammatory process leading to Type 2 diabetes	Dr.Sudeep Ghosh
8. Padmavathi I.J.N. (2004)	Role of maternal chromium status in the development of insulin resistance in the offspring	Dr.Raghunath M.
9. Satyanarayana B. (2004)	Biological significance of phytoferritins	Dr.Madhavan Nair K.
10. Sreenivasulu K. (2004)	$CaCo_2$ cell as a model to study bioavailability, mechanism of absorption and cytoprotective effects of zinc	Dr.Madhavan Nair K.
11. Vasuprada I. (2005)	Bio-response of a model CaCo-2 cell system of iron and zinc	Dr.Madhavan Nair K.

Research Scholar/staff	Title of thesis	Guide
12. Shashikiran G (2005)	*In vitro* regeneration of the insulin secreting cells from the adult pancreatic ductal epithelial cells (progenitors/stem cells) - The role of specific nutrients	Dr.Vijayalakshmi V.
13. Sheril Alex (2005)	PUFA-rich oil diet supplementation on body weight regulation of obese rat model of WNIN/GR-Ob strain: A nutrient-gene interaction stud	Dr.Vajreswari A.
14. Rajkumar (2005)	Charecterization and differentiation of pancreatic progenitor/stem cells (Nestin positive cells) to insulin secreting cells-the role of specific micronutrients	Dr.Vijayalakshmi V.
15. Manisha Ganeshan (2005)	Foetal origins of adiposity and insulin resistance: Role of peri/postnatal manganese status	Dr.Raghunath M.
16. Vara Prasad SSS (2005)	Role of 11?-HSD1 in pathogenesis of obesity and insulin resistance in WNIN/GR-Ob and WNIN/Ob restrains	Dr.Vajreswari A.
17. Sainath P.B (2005)	Insulin, insulin receptor and its signaling mechanism(s) in the brain and insulin sensitive target organs in the WNIN/ob and WNIN/GR-ob rats	Dr.Raghunath M.
18. Pratibha B. (2005)	Immune status of WNIN mutant rats with reference to leptin and obesity	Dr.Giridharan N.V.
19. Sreevani M. (2005)	Understanding & dissecting the role of resistin in etiology of insulin resistance using obese rat model	Dr. Nasreen Z. Ehtesham
20. Yadagiri Reddy P. (2006)	Biochemical studies on obesity induced cataractogenesis using WNIN obese rat model	Dr.Bhanuprakash Reddy G.
21. Naga Bala Shankara rinivas P. (2006)	Studies on the significance of ?-crystallin heteropolymer in the eye lens	Dr.Bhanuprakash Reddy G.
22. Anand Kumar K. (2006)	Maternal vitamin B12 restriction induced changes in body adiposity, hyperglycemia and insulin resistance in WNIN rat offspring: Molecular basis of the changes	Dr.Raghunath M.

Research Scholar/staff	Title of thesis	Guide
23. Priyanka Shanker (2006)	Study on high fluoride and low calcium on bone metabolism in rats: biochemical mechanisms	Dr.Arjun L. Khandare
24. Y. Srinivasa Reddy (2006)	Effect of environmental lead exposure on infection and immunity in undernutrition	Dr.Kalpagam Polasa
25. Little Flower Augustine (2007)	Stress allostatic load and micronutrient status among higher secondary students: Impact of dietary advise	Dr. Madhavan Nair K.
26. Mr.P.Muthenna (2007)	Characterization of active principles from dietary sources, Mechanism of action of Aldose Reductase inhibitors and protein glycation inhibition	Dr. Bhanuprakash Reddy G.
30. Ms.Swarnim Gupta (2008)	Dietary diversification of Indian vegetarian diet to improve iron bioavailability : Studies using Caco-2 cell model	Dr. Madhavan Nair K.
31. Mrs.Soundarya (2008)	Establishment of propoyable cell lines from adult adipose tissue of WNIN mutant rats (WNIN Ga/Ob and Ob/Ob)	Dr.Vijayalakshmi V.
32. Deethu Sara Vergheese (2008)	Assessment of body composition in Indian females using different techniques	Dr.Venkataramana Y.
33. Mr. B. Sankar Anand (2009)	Role of T-cells and secreted cytokines in insulin resistance and obesity	Dr.Sudip Ghosh
34. Mr. Ramesh Athe (2009)	Meta analysis on “Micronutrient food fortification and its effect on health, social and economic factors” – A statistical model building	Dr. Vishnuvardhan Rao M.
35. Mr. Nimgulkar Chetan Chandrakant (2009)	Evaluation of herbs/nutraceuticals products as anti-atherosclerotic agent	Dr. Dinesh Kumar B.

AWARDS/ HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Award/Honour
Dr.C.Vijayakumar Reddy	Best Poster Award for the paper entitled "Antioxidant activity of some common Indian fruits", at the Scoiety for Free Redaical Research (SFRR)-India 2008 Conference, organized by All India Institute of Medical Sciences, New Delhi.
Dr.K.Madhavan Nair	Selected as Member of the National Academy of Sciences, India.
Dr.G.Bhanuprakash Reddy	Scopus Young Scientist in Medicine" for the year 2008 by Elsevier (Science & Technology), Noida, India.
Dr.P.Suryanarayana	Association for Research in Vision and Opthalmology (ARVO) – International Travel Award to attend the annual meeting of ARVO, at Fort Lauderdale, Florida, USA.
Dr.SSYH Qadri	Qualified the founding IVCP certification examination conducted by Indian College of Veterinary Pathologists, Indian Veterinary Research Institute, Bareilly, India.
Mr.GM.Subba Rao	Awarded JN Bose memorial award for Community Nutrition in the 41st National Conference of Indian Dietetic Association, for the paper entitled "Nutrition and Food Safety in School Science Curricula – A Content Analysis".

PARTICIPATION OF SCIENTISTS IN INTERNATIONAL MEETINGS/ WORKSHOPS/CONFERENCES/ SEMINARS/ TRAINING

Date	Name of the Scientist	Conference/Meeting/Workshop/Seminar
2008		
April 27 – May 1	Dr.P.Suryanarayana & Ms.Megha Saraswat	International Conference on "Association for Research in Vision and Opthalmology", at Florida, USA. They presented papers on "Inhibition of aldose reductase, protein glycation and delay of diabetic cataract in rats by rutin" and "Inhibition of protein glycation by dietary agents: Implications in the prevention of diabetic ocular complications" respectively.
May 1, 2008 – April 1, 2009	Dr.P.Suryanarayana	DST-BOYSCAST-Fellowship (2007-08), at the Department of Ophthalmology, Washington University, St.Louis and Rocky Mountain Lions Eye Institute, University of Colorado, Denver, USA
July 19-23	Mr.PNBS.Srinivas	Meeting of the Protein Society to be held at San Deigo, USA. Presented a paper on "Significance of α-crystallin heteropolymer with respect to its molecular chaperone function".
Sept. 20, 2008 – Sept. 11, 2009	Dr.J.J.Babu Geddam	Participated in the Netherlands Fellowship Programme and obtained Masters in Public Health from Royal Tropical Institute, VU University, Amsterdam, Netherlands.
Sept. 30 - Oct.3	Dr.GNV.Brahmam	WHO Meeting on the "Dietary management of moderate malnutrition" at Geneva, Switzerland.
Nov. 11-13	Dr.B.Sesikeran	First Plenary Meeting of ISO/TC 34/SC 16 of Bureau of Indian Standards (BIS), at Rosemont (Chicago), IL, USA.
2009		
Feb. 14-22	Dr.M.Raghunath	Workshop on Medical Research Communication, being organized by PRIMO Scientific Corporation, at Singapore.

WORKSHOPS/ CONFERENCES/ SEMINARS/TRAINING PROGRAMMES HELD AT NIN

I. WORKSHOPS/CONFERENCES/SEMINARS

1. DGHS Sub Group meeting on "Monosodium glutamate – for removal of labeling requirements under PFA rules, 2008" (April 21, 2008).
2. DGHS-CCFS meeting on "National survey on use of synthetic colours in sweets and beverages" (April 21, 2008).
3. Symposium on "GM Food Safety Assessment in India: Taking Stock and Planning for the Future", organized by Indian Council of Medical Research in association with Biotech Consortium India Ltd. and South Asia Biosafety Program (July 7, 2008).
5. Meeting of the Scientific Advisory Committee of NIN/FDTRC/NCLAS (Aug. 12-14, 2008).
6. Brain Storming Session on "Harmonization of allergencity assessment protocols for conventional and novel foods & food products" sponsored by DBT (Aug.20-21, 2008).
7. Women's Scientist Scheme – A (WOS-A), The 3rd meeting of Subject Experts Committee on Life Sciences, sponsored by DST (Aug.29-30, 2008).
8. GLP Workshop on "Basic principles & practices of Good Laboratory Practice (GLP)" (Sept. 4, 2008).
9. Symposium on "Role of Nutrition in Brain Development and Function" (Sept.15, 2008).
10. Meeting of the "9th WHO Southeast Asia Nutrition Research-cum-Action Network" (Sept. 24-26, 2008).
11. Workshop on "Basic Principles & Practices of Good Laboratory Practices", in association with National GLP Compliance Monitoring Authority, Department of Science & Technology, Ministry of Science & Technology, New Delhi (Nov. 26, 2008).
12. 41st National Conference of Indian Dietetic Association. Pre-Conference Workshop on "Preparing Tomorrow's Dietitians" (Dec. 4, 2008).

II. TRAINING PROGRAMMES

1. Orientation Training Programme in "Methodology of Nutrition Assessment" was conducted to the scholars of Anthropological Survey of India, Kolkata (March 31 – April 11, 2008).
2. Laboratory Animals Technicians Training Course. Nine participants underwent the training programme (June 15 – July 25).
3. 36th Annual Training Course on Endocrinological Techniques and their Applications. Ten candidates participated in the course (Aug 16 – Sept. 28, 2008)
4. An ad-hoc training programme was conducted for the staff of Foundation for Revitalization of Local Health Traditions (FRLHT) in "Coupled invitro digestion/Caco-2 cell based iron bioavailability screening method" (Sept. 15-24, 2008).
5. XXXXVI Post-Graduate Certificate Course in Nutrition. Twelve candidates from different States of the country participated in the Course including two candidates sponsored by WHO (Jan. 5-March 20, 2009).

SERVICES RENDERED TOWARDS INCOEME GENERATION

1. PATHOLOGY SERVICES

During the year, a total income of Rs. 3,76,640/- was generated from various projects of institute's preclinical toxicology and surgical pathology and cytology samples.

2. TRAINING PROGRAMMES

By admitiing 12 unsponsored private candidates and two WHO sponsored candidates to the regular training courses, an amount of Rs.2,50,000/- was generated.

SCIENTIFIC PUBLICATIONS

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1. Arlappa N, Laxmaiah A, Balakrishna N, Harikumar R, Brahmam GNV : Clinical and sub-clinical vitamin A deficiency among rural pre-school children of Maharashtra, India. Ann Hum Biol. 35 : 606-614, 2008.
2. Bamji MS, Murthy PVSS, Livia Williams, Vishnuvardhana Rao M : Maternal nutritional status & practices & perinatal, neonatal mortality in rural Andhra Pradesh, India. Indian J Med Res. 127 : 44-51, 2008.
3. Bhanuprakash Reddy G, Satyanarayana A, Balakrishna N, Radha A, Viswanath K, Mark Petrash J : Erythrocyte aldose reductase activity and sorbitol levels in diabetic retinopathy. Molecular Vision. 14 : 593 – 601, 2008.
4. Bharati Kulkarni, Veena Shatrugna; Nagalle B, Ajeya Kumar P, Usha Rani, Chandrakala Omkar A : Maternal weight and lean body mass may influence the lactation related bone changes in young under nourished Indian women. Br J Nutr. (Epub.) Oct : 1-7, 2008.
5. Bhaskarachary K, Ananthan R, Longvah T: Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. Food Chemistry. 106 : 85-89, 2008.
6. Brahmam GNV, Venkaiah K, Hemalatha R, Hari Kumar R, Srinivasan K, Shiva Prakash M, Paranjape RS, Gupte MD, Lakshmi Ramakrishnan, Anjali Kohli, Ramesh BM : Sexual practices, HIV and sexually transmitted infections among self-dentified men who have sex with men in four high HIV prevalence states of India. AIDS. 22 (suppl.5) : S45 – S57, 2008.
7. Christina M, Nythus Dhillon, Per Pinstrup-Andersen, Jere D Haas, Nagalla Balakrishna, Brahmam GNV : The Effect of bioforified rice and wheat in India's food supply on dietary bioavailable iron. The FASEB J. 22 : Ib770, 2008. (Abstract).
8. Fernandez S, Balakrishna N, Shahnaz Vazir, Peggy Bently P, Johnson S, Engle P : Maternal self esteem and locus of control relates to the quality of young children's environment (Home) in rural Andhra Pradesh, India : Research and Policy implications. Int J Early Childhood. 40 : 85 – 99, 2008.
9. Gajre NS, Fernandez S, Balakrishna N, Shahnaz Vazir : Breakfast eating habit and its influence on attention – concentration, immediate memory and school achievement. Indian Pediatrics. 45 : 824-828, 2008.
10. Grace Maria Antony, Laxmaiah A : Human development, poverty, health and nutrition situation in India. Indian J Med Res. 128 : 198-205, 2008.
11. Hemalatha R, Ramalakshmi BA, Quadri SSYH, Balakrishna N, Annapurna VV, Sesikeran B : Intrauterine growth restriction in term women with histologic chorioamnionitis. Res J Obstet Gynecol. 1 : 18 – 24, 2008.

12. Ira Surolia, Sharmistha Sinha, Debi Prasad Sarkar, Yadagiri Reddy P, Bhanuprakash Reddy G, Avadhesha Surolia : Concurrence of Danish dementia and cataract : Insights from the Interactions of dementia associated peptides with eye lens a – crystallin. PLoS ONE. 3 : 1-9, 2008. (Open Access).

13. Jeyakumar SM, Vajreswari A, Giridharan NV : Vitamin A regulates obesity in WNIN/Ob obese rat ; independent of stearoyl – CoA desaturase - 1. Biochem Biophys Res Commun. 370 : 243-247, 2008.

14. Kamarthapu V, Rao KV, Srinivas PN, Reddy GB, Reddy VD : Structural and kinetic properties of bacillus subtilis S-adenosylmethionine synthetase expressed in Escherichia coli. Biochim Biophys Acta. – Proteins Proteom 2008, June 19 (Epub).

15. Khandare AL, Siruguri V, Rao A, Venkaiah K, Reddy G, Rao GS : Diet and nutrition status of children in four tribal blocks of Thane district of Maharashtra, India (Nutrition Status of Children). Pakistan J Nutr. 7 : 485-488, 2008.

16. Kumar PA, Reddy PY, Srinivas PN, Reddy GB : Delay of diabetic cataract in rats by the antiglycating potential of cumin through modulation of alpha-crystallin chaperone activity. J Nutr Biochem. 2008, Sep. 10 (Epub).

17. Megha Saraswat, Muthenna P, Suryanarayana Rao P, Mark Petrah J, Bhanuprakash Reddy G : Dietary sources of aldose reductase inhibitors : prospects for alleviating diabetic complications. Asia Pac J Clin Nutr.17 : 2008.

18. Megha Saraswat, Yadagiri Reddy P, Muthenna, Bhanuprakash Reddy G : Prevention of non-enzymatic glycation of proteins by dietary agents: prospects for alleviating diabetic complications. Br. J Nutr.

19. Nagajyoti PC, Dinakar N, Prasad TNVKV, Suresh C, Damodharam T : Heavy metal toxicity; Industrial effluent effect on groundnut (Arachis hypogaea L.) seedlings. J Appl Sci Res. 4 : 110 – 121, 2008.

20. Nagajyoti PC, Dinakar N, Udaykiran Y, Prasad K, Suresh C, Damodharam T : Effect of biomass power plant effluent on biochemical parameters of Arachis hypogaea L. Asian J Chem. 20 : 5489 – 5496, 2008.

21. Nirmala K, Prasanna Krishna T, Polasa K : Alterations in antioxidant status of rats following intake of ginger through diet. Food Chemistry. 106 : 991-996, 2008.

22 Nirmala K, Prasanna Krishna T, Polasa K : Inhibition of induced micronuclei formation in human lymphocytes by Ginger. Int J Cancer Res. 4 (1) : 12-19, 2008.

23. Padmaja Rambabu J : The reality of food colours. Times Food Processing Journal. Dec-Jan 2009, p.42.

24. Panpatil VV, Kalpagam Polasa : Assessment of stevia (Stevia rebaudiana - natural sweetener : A Review. J Food Sci Technol. 45 : 467 – 473, 2008. (I.F. 0.107)

25. Pratima Rao, Sudershan RV : Risk assessment of synthetic food colours : a case study in Hyderabad, India. Int J Food Safety, Nutr & Public Health. 1 : 68-87, 2008.

26. Raghu P, Madhavan Nair K, Sunanda K, Sreenivasulu K, Tippeswamy Gowda T : Ferric reductase activity of low molecular weight human milk fraction is associated with enhanced iron

solubility and uptake in Caco-2 cells. Biochem Biophys Res Commun. 374 : 369 – 372, 2008.

27. Ramesh BM, Stephen Moses, Reynold Washington, Shajy Isac, Mohapatra B, Mahagaonkar SB, Adhikary R, Brahmam GNV, Paranjape RS, Subramanian T, Blanchard JF : Determinants of HIV prevalence among female sex workers in four south Indian states : analysis of cross-sectional surveys in twenty-three districts. AIDS. 22 (Suppl 5) : S35 – S44, 2008.

28 Ranganathan S, Sesikeran B : Development of the Double-Fortified Salt from the National Institute of Nutrition. Comprehensive Rev Food Sci, Food Safety. 7:390 – 396, 2008.

29. Sesikeran B, Vasanthi Rao S : Constantly evolving safety assessment protocols for GM foods. Asia Pac J Clin Nutr. 17 (S1) : 241 – 244, 2008.

30. Sanjay Kinra, Rameshwar Sarma KV, Ghafoorunissa, Vishnu Vardhana Rao M, Radhakrishnan R, Viswanathan Mohan, Ian B Wilkinson, John R Cockroft, George Davey Smith, Yoav Ben-Shlomo : Effect of integration of supplemental nutrition with public health programmes in pregnancy and early childhood on cardiovascular risk in rural Indian adolescents : long term follow-up of Hyderabad nutrition trial. Br Med J. 29th July 2008. Online.

31. Sreenivasulu K, Raghu P, Ravinder P, Madhavan Nair K : Effect of dietary ligands and food matrices on zinc uptake in Caco-2 cells: Implications in assessing zinc bioavailability. J Agric Food Chem. 56 : 10967-10972, 2008.

32. Srinivas PNBS, Reddy PY, Reddy GB : Significance of alpha-crystallin heteropolymer with a 3:1 alphaA/alphaB ratio: chaperone-like activity, structure and hydrophobicity. Biochem J. 414 : 453-460, 2008.

33. Subramanian T, Gupte MD, Paranjape RS, Brahmam GNV, Lakshmi Ramakrishnan, Adhikary R, Kangusamy B, Thomas BE, Srinivasan K, Girish CPK : HIV, sexually transmitted infections and sexual behaviour of male clients of female sex workers in Andhra Pradesh, Tamil Nadu and Maharashtra, India : results of a cross-sectional survey. AIDS. 22 (Suppl 5) : S69-S79, 2008.

34 Sudershan RV, Subba Rao GM, Pratima Rao, Vishnu Vardhana Rao M, Kalpagam Polasa : Food Safety related perceptions and practices of mothers – A case study in Hyderabad, India. Food Control. 19 : 506-513, 2008.

35. Sudershan, RV, Subba Rao GM, Pratima Rao, Vishnu Vardhana Rao M, Kalpagam Polasa : Knowledge and practices of food safety regulators in Southern India. Nutr Food Sci. 38 : 110 –120, 2008.

36. Sudershan Rao V, Subba Rao GM :Junk food–Are we miscommunicating ? Ind J. Nutr Dietet. 45 : 155 – 159, 2008.

37 Vajreswari A, Jeyakumar SM: Retinoids : Impact on adiposity, lipids and lipoprotein metabolism. Recent Patents on Endocrine, Metabolic & Immune Drug Discovery. 2 : 109-122, 2008.

38. Veena Shatrugna, Bharati Kulkarni, Ajay Kumar P, Balakrishna N, Usha Rani K; Chennakrishna Reddy G, Narasimha Rao GV : Relationship between women's occupational work and bone health – a study from India. British J Nutr. 99 (6) : 1310-1315, 2008.

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