वार्षिक प्रतिवेदन
Annual Report
2012 - 13

राष्ट्रीय पोषण संस्थान
National Institute of Nutrition
(भारतीय आयुर्विज्ञान अनुसंधान परिषद)
(Indian Council of Medical Research)

Annual Report
2012-13

सत्यमेव जयते

डॉ.विश्व मोहन कटोच
एम डी, एफ एन एससी, एफ ए एम एस, एफ ए एससी, एफ एन ए
सचिव, भारत सरकार
(स्वास्थ्य अनुसंधान विभाग)
स्वास्थ्य एवं परिवार कल्याण मंत्रालय एवं
महानिदेशक, आई सी एम आर

Dr. Vishwa Mohan Katoch
MD, FNASc, FAMS, FASc, FNA
Secretary to the Government of India
(Department of Health Research)
Ministry of Health & Family Welfare &
Director-General, ICMR

भारतीय आयुर्विज्ञान अनुसंधान परिषद
(स्वास्थ्य अनुसंधान विभाग)
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
वी. रामलिंगस्वामी भवन, अंसारी नगर
नई दिल्ली - 110 029 (भारत)

Indian Council of Medical Research
(Department of Health Research)
Ministry of Health & Family Welfare
V. Ramalingaswami Bhawan, Ansari Nagar
New Delhi - 110 029 (INDIA)

MESSAGE

India has succeeded in tackling severe forms of undernutrition in children through the effective implementation of national nutrition programmes and streamlining of Public Distribution System to some extent. However, the challenge of ensuring total nutrition security, especially among the disadvantaged sections of our population still remains a distant dream. Poor Infant and Young Child Feeding Practices, including poor breastfeeding practices prevalent in our communities are compounding the problem further. Obesity has also emerged as other type of malnutrition which is equally difficult to prevent and manage. By and large we have not succeeded in understanding and handling nutritional transition.

A majority of people living in rural areas, urban slums and certain tribal pockets of the country are found to be highly vulnerable to several micronutrient deficiency disorders. Vitamin A, iron and iodine deficiencies affect them with deleterious impact and diminish the quality of their lives. The recent report of the third repeat survey of the National Nutrition Monitoring Bureau (NNMB) has underscored the immediate need for the promotion of nutrition security among these nutritionally vulnerable groups. The studies conducted by NNMB say that diet related non-communicable diseases like hypertension and diabetes also affect those living in non-urban areas in significantly large numbers and this indeed is a cause of concern.

It is time we redefine our research priorities with more vigour and proper direction and meet the immediate needs of our communities, in both the realms of health and nutrition. Effective nutrition education programmes, need based nutritional interventions along with appropriate inter-sectoral coordination between all the stakeholders working for the promotion of nutrition security in the country are certain practical measures, which need to be undertaken on war footing now. I am happy that NIN has started playing major role in supporting the fight against malnutrition by research focused on high burden districts.

I implore all the scientists to rise to the occasion and be visionaries in their research endeavors. We, as the members of scientific community are responsible for better health and nutritional status of our people. The need for translational and implementation research was never felt earlier with such increased intensity as it is being felt now. NIN and other scientists working on nutrition need to concentrate on understanding our old healthy practices and partner with agriculture scientists for producing crops accordingly.

I wish a great success to NIN and its partners.

(Vishwa Mohan Katoch)

Tele.: (Off.) +91-11-26588204, 26589620; Fax (Off.): +91-11-26588662, E-mail: secydhr@icmr.org.in; dg@icmr.org.in

CONTENTS

SCIENTIFIC STAFF

DIRECTOR
B.Sesikeran, MD
(Till August 31, 2012)

Kalpagam Polasa, MSc, PhD, MBA
(SCIENTIST 'F' & DIRECTOR-INCHARGE)

CLINICAL
R. Hemalatha, MD
(Scientist 'E')
Bharati Kulkarni, MBBS, DCH, MPH
(Scientist 'E')
K. V. Radhakrishna, MBBS, DCH
G. Jagjeevan Babu, MBBS
Sadhana Gupta, MSc, PhD
Raja Sriswan Mamidi, MBBS

PATHOLOGY
P. Uday Kumar, MD
(Scientist 'F')
SSYH. Qadri, MVSc
M.V.Surekha, MD

MICROBIOLOGY & IMMUNOLOGY
R. Hemalatha, MD
(Scientist 'E')
M. Shiva Prakash, MSc, PhD
Devraj J. Parasannanavar, MSc, PhD
A.Vijayendrachary, MSc (RF)
N.Himaja, MSc (RF)
N.Padmaja, MSc (RF)

LIPID CHEMISTRY
A. Vajreswari, MSc, PhD
(Scientist 'F')
S.Ahmed Ibrahim, MSc, PhD
S. M.Jeyakumar, MSc, MPhil, PhD
P. Sujatha, MSc, PhD
Anupama Tyagi, MSc (RF)
S.Anil, MSc (RF)
Ch. Anuradha, MSc (RF)
V. Anantha Krishna, MSc (RF)
J.Sugeetha, MSc (RF)

STEM CELL BIOLOGY
V.Vijayalakshmi, MSc, PhD
(Scientist 'E')
C. Suresh, MSc, PhD
A. Rajanna, Msc
M.Soundarya, MSc (RF)
Himadri Singh, MSc (RF)
K Sowmya, MSc (RF)

MOLECULAR BIOLOGY
Sudip Ghosh, MSc, PhD
Sanjay Basak, MSc, PhD
M.Srivani, MSc (RF)
B. Shankar Anand, MSc (RF)
G. Venkateswarulu, MSc (RF)
K. Sandeep Kumar, MSc (RF)

MICRONUTRIENT RESEARCH
K. Madhavan Nair, MSc, PhD
(Scientist 'F')
P. Raghu, MSc, PhD
P. Ravinder, MSc, PhD
Little Flower Augustine, MSc (RF)
Swarnim Gupta, MSc (RF)
A. Kiran Kumar, MSc (RF)
N. Pallavi, MSc (RF)
Y. Sravanthy, MSc (RF)
P.Ravindranath, MSc (RF)
M. Purna Chandra, MSc (RF

WORK PHYSIOLOGY
Y. Venkataramana, MSc, PhD
(Scientist 'E')
Deethu Sara Varghese, MSc (SRF)

FOOD CHEMISTRY
T. Longvah, Msc
(Scientist 'F')
K. Bhaskarachary, MSc, PhD, PGDN&DM
J.Sreenivasa Rao, MSc
S.Devendra, MSc, Mphil, PhD
R.Ananthan, MSc, PhD
J. Vahini, MSc (JRF)
Naveena Natrajan, MSc (JRF)
Daniella Chyne, MSc (JRF)

ENDOCRINOLOGY & METABOLISM

M. Raghunath, MSc, PhD
(Scientist 'F')
Ayesha Ismail, MSc, PhD
Jitendra Kumar Sinha, MSc (SRF)
Shampa Ghosh, MSc. MPhil (SRF)
Mehraj-ud-din, MSc (SRF)
N.Bindu, MSc (JRF)
Anju Elizabeth Joseph, MSc (JRF)
K.Nagabhushan Reddy, MSc (JRF)

OCULAR BIOCHEMISTRY

G. Bhanuprakash Reddy, MSc, PhD
(Scientist 'E')
P. Suryanarayana, MSc, PhD
N.Saravanan, MSc, PhD
V.Sudhakar Reddy, MSc (SRF)
Sara Sarin Jose, MSc (SRF)
K. Ashok Reddy, Msc (RF)
Madhusudhan A. Patil, MSc (SRF)
S.Vishwaraj, MSc (JRF)
Sneha Jakhotia, MSc (RF)
K.Shruthi, MSc (RF)
T.Shalini, MSc (RF)

FIELD DIVISION

A. Laxmaiah, MBBS, MPH
(Scientist 'F')
R. Harikumar, MBBS, DPH
N. Arlappa, MBBS
I.I.Meshram, MBBS, MD(PSM)
K. Mallikharjuna Rao, MSc, PhD
M.S. Radhika, MSc, PhD
Sylvia Fernandez Rao, MA, PhD
Ch. Gal Reddy, Msc

BIOSTATISTICS

K. Venkaiah, Msc
(Scientist 'F')
M. Vishnuvardhan Rao,
MSc, PhD, MTech (IT)(Scientist 'E')
N. Balakrishna, MSc, PhD
Ramesh Athe, Msc, Mphil

EXTENSION & TRAINING

D. Raghunatha Rao,
Msc, PhD, PG Dip. FNS (Scientist 'E')
T. Vijaya Pushpam, MA, MPhil, PhD
M.Maheshwar,
MCOM,MA,MCJ, LLB, MPhil

G. M. Subba Rao,
MA, PGDJ, PGDT, PhD
K. Damayanthi, MSc, PhD

INSTRUMENTATION

V.Vijayalaxmi, MSc, PhD
(Scientist 'E')

FOOD & DRUG TOXICOLOGY RESEARCH CENTRE (FDTRC)

Director (Till August 31, 2012)
B.Sesikeran, MD

Kalpagam Polasa, MSc, PhD, MBA
(Scientist 'F' & Director In-charge)

FOOD TOXICOLOGY

Arjun L Khandare, MSc, PhD
(Scientist 'E')
S.N.Sinha, MSc, PhD
J. Padmaja, MSc, PhD
V. Sudershan Rao, MSc, PhD
S. Vasanthi, MSc, PhD
P. Amrutha Rao, MBBS, DPH
Priyanka Shankar, MSc (SRF)
Agatha Betsy, MSc (SRF)
N.Aparna, MSc (JRF)
M.Ankulu, MSc (JRF)
Alekhya, MSc (JRF)

DRUG TOXICOLOGY

M.P. Rajendra Prasad,
MBBS, MSc (AN), PhD, (Scientist 'E')
B. Dinesh Kumar, MSc (Pharma), PhD
(Scientist 'E')
Chetan Nimgulkar, MSc (SRF)
M. Chalamaiah, MSc (SRF)
V.Varsha, MSc (JRF)

NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

Director (Till August 31, 2012)
B.Sesikeran, MD

P. Suresh Babu, MVSc
(Scientist 'F')
N. Hari Shanker, MSc, PhD
K.Rajender Rao, MSc, PhD
N.Muralidhar, MSc (JRF)

ADMINISTRATIVE STAFF

(MINISTERIAL & SECRETARIAL)

SENIOR ADMINISTRATIVE OFFICER
L.Chandrasekhara Rao

ADMINISTRATIVE OFFICER
N. Sitaramanjaneyulu
A.Suryaprakash Rao
M. Ashok Raj

ACCOUNTS OFFICER
G. Krishna Reddy

SECTION OFFICERS
M. Vijay Kumar
G. Ramakrishna
Abdul Rasheed Shaik
Latha Kumarsswamy
N.Muralikrishna
M.Siva
P.Dhanasekharan

PRIVATE SECRETARIES
Ch. Madhulatha
P.Venugopal
Sudha Srinivasan
D.V.Laxmi Rani

ASSISTANTS
V.Laxminarayana
R.C.Padmini Mohan
D.Venkateswarlu
K.Ch.Ramayya Dora
T.Satyanarayana
K.Sivarami Reddy
M.Rajagopala Chary
M.K.Koteshwara Reddy
V.Elisha
Alice Mary
D.Sunil
A.Narayana Reddy
M.Babu
C.Kalavathi
D.Seetharamaiah

PERSONAL ASSISTANTS
G.Hanumantha Rao
Malini V. Rao
G.Prashanthi
V.Swayam Prabha
K.Sailaja

UPPER DIVISION CLERKS
E. Syama Sundari
Shakila Banu
G.R. Srinivas
K.Jayamma
P. Prabhavathi
Mohd. Iliyas
Shaik Jamaluddin
C.Prabhu
Mini Pramod
T.Anuradha Jayalaxmi
A.Narsing Rao
G.S.Gautami
A.Satyanarayana Prasad
Y.Bala Narayana

STENOGRAPHERS
G. Mahesh Kumar

LOWER DIVISION CLERKS
M. Rekha
D. Ramanjaneyulu
G.Y.Anita
A. Venkataramana
U. Somayya
M. Raghuram

RECEPTIONIST-CUM-TELEPHONE OPERATOR
M. Jawahar Joshua

LIBRARY STAFF

Asst. Library Information Officer
B.Narayana
Library Information Assistant
Prakash Kulkarni
Library Clerk
Ungarala Naidu

TECHNICAL STAFF

Technical Officer "B" (Tech. staff)

1. Ramachander Chaugule
2. S. Ananda Rao
3. Ch. Gal Reddy
4. Anil kumar dube
5. Sharad kumar
6. M. Ravindranath

Technical Officer "B" (Engg. Support staff)

1. Bandam Ramulu

Technical Officer "A" (Tech. staff)

1. G. Amarendra Reddy
2. A. Kasiviswaraja Mouli
3. V. Satish Babu
4. K.Nirmala
5. M.Vijayalakshmi
6. K.Yadamma
7. K. Swarupa rani
8. M.Satyavani
9. Virendra Vasant Panpatil
10. M. Krupadanam
11. R. Naveen Kumar
12. B.Narahari
13. Laxmi Rajkumar
14. Vani Acharya
15. D. Sreeramulu
16. C. Maniprabha
17. Abhay Kumar
18. K. Vinod Reddy
19. V. Vikas Rao
20. Kasinath Lingreker
21. R. Radhakrishna Sarma
22. V.Radhakrishna Rao
23. S. Prabhakar
24. D. Pandu Ranga Vittal
25. K. Nageswara Rao
26. Ch. Nagambika Prasad
27. S. Lopamudra
28. S. Hemalatha
29. Amulya Rao
30. G. Shanker Rao
31. K. Srinivasa Rao
32. B. Pothu Raju
33. Korra Mangthya
34. R. Ravindar Naik
35. D.Vijaya Bhaskara Rao
36. P. Ajey Kumar
37. Saleem Shariff
38. G.V. Narasimha Rao
39. N. Sreenivasa Chary

Technical Officer "A" (Engg. Support staff)

1. Mota Chandrasekhara Rao
2. N. Hanumantha Rao
3. P. Moses Ashok Kumar
4. U.D. Awasthi
5. G. Mohan Rao
6. G.B. Walter
7. L. Vijaya durga

Technical Assistant (Tech. staff)

1. P.Krishnaswamy
2. G. Chenna Krishna Reddy
3. K. Narasimha Reddy
4. T. Nagasekhara Rao
5. Ch. Ranga Rao
6. V.V. Narasimha Reddy
7. K. Sharada
8. P. Sailaja
9. D. Prem Raj
10. B.R. Annapurna
11. Ch. Hanumantha Reddy
12. Ch. Krishna
13. V. Bhasker
14. P. Yadagiri Reddy
15. M. Seshacharuyulu
16. S.A. Brinda
17. K. Subash
18. K. Vasudev
19. M. Srinivas
20. K. Suryam Reddy
21. S. Laxman

Technical Assistant (Engg. Support staff)

1. B. Om Prakash
2. B. Venkateswara Rao
3. N. Satyanarayana
4. G. Janardhan
5. M. Asaithurai

6. Micheal Fernandez
7. A. Anjaiah
8. T. Shyam Sunder
9. Joseph Vijaykimar
10. YVL Narasimha Rao
11. Mohd. Younus
12. G.P. Narender

Driver (Special Grade)

1. P. Mahender

Technician "C" (Tech. staff)

1. Narottam Pradhan
2. B. Tulja
3. K. Swatantra Rao
4. P. Madhusudhana Chary
5. R. Raghunath Babu
6. K. Usha Rani
7. C. Sai Babu
8. P. Satish Babu
9. C. Janardhan
10. G. Govindarajulu
11. P.S. Prashanthi
12. S.P.V. Prasad
13. K. Sree Ramakrishna
14. P. Anitha Chauhan
15. G. Madhavi
16. B. Giri Babu
17. P.S. Rama Rao
18. G. Venkataraji Reddy
19. J. Narasimhulu
20. S. Ashok
21. G.I. Stephen
22. G.A. Rabbani

Technician "C" (Engg. Support staff)

1. K. Pavan Kumar
2. Syed Jalaluddin Hussaini
3. R. Sahadeva
4. J. Kumaraswamy
5. A.I. Goverdhan
6. K. Srenivasa Raju
7. N. Narasimha
8. Purnachandra Beshra
9. P. Dasarath
10. S. Devendran
11. Ramavath Ramsingh
12. Sriramulu Naidu
13. E. Srinivas
14. V. Bhuvaneswaran
15. Polishetty Naidu
16. P. Narendra Kumar

Technician "B" (Tech. Staff)

1. E. Sammi Reddy
2. K. Balaji
3. M. Sripal Reddy
4. N. Peddi Reddy
5. Y. Agreepa Raju
6. P. Sashidharan Pillai
7. Y. Salaiah
8. Gandamalla Narasimha
9. M. Venkatesh
10. R. Yadagiri
11. P. Bheem Shanker
12. J. Pochaiah
13. M. Balram
14. S. Chandraiah
15. B. Nagender rao
16. Prabhu Raj
17. P. Nagabhashunam
18. Nigala Yadagiri
19. E. Krishna
20. Bommaka Srinu
21. Neelakanta
22. L. Dasu
23. D. Dasaratha
24. J. Nageswara Rao
25. C. Chandramouli
26. Abdul Sattar
27. N. Rajaiah
28. K. Rama Rao
29. V. Rajkumar

Technician "B" (Engg. Support staff)

1. B. Bal Reddy
2. J. Bhujender
3. K. Parthasarathy

Technician 'A' (Engg. support staff)

1. D. Ravinder
2. N. Ramesh Kumar
3. Mahender Singh Jadav

Driver (Grade – I)

1. Zahid Ali Khan
2. K. Krishna

3. V. Kondaiah
4. Syed Mohd. Ali

Driver (Grade – II)
1. D. Amruthanathan
2. K. Jangaiah

Junior Staff Nurse
1. G. Rajakumari
2. B.V. Nancharamma

Nursing Attendant

1. G. Tulasi Bai
2. V. Aruna Reddy
3. E. Sheela
4. G. Vijayalakshmi
5. R.Rajyalakshmi
6. Govada Bhavani
7. Valentina Teriscova
8. D.Swarupa

Senior Cook (Isolated Post)
1. H.S. Ramu

Attendant (Services)
1. Y. Veeraiah
2. K.B. Raju
3. G. Bichapathi
4. R. Pochaiah
5. C. Shankaraiah
6. Manga Narasaiah
7. B. Balanarasaiah
8. M. Eshwar
9. Abdul Bhasid
10. G. Eswaraiah
11. G. Viswanatham
12. M. Suresh
13. Mohd. H. Yousuf
14. Mohd. Abdul Khader
15. Bondi Ramulu
16. J. Yadagiri
17. Syed Mohd. Iqbal
18. C. Rajaiah
19. Mabbu Ramulu
20. V. Shanker
21. Kompally Pochaiah
22. A. Narasaiah
23. Mukkera Krishna
24. Mohd. Mehboob
25. J. Lakshmaiah
26. K. Rajaiah
27. P.V. Poulous

3. D. Threessamma
4. D. Rani

Auxiliary Nurse Midwife

1. K. Venkataramana
2. S. Rojamani
3. K. Santhosham
4. Ch. Anitha

Attendant (Services) Contd..
28. Manupathi Bikshapathi
29. Dhanavath Saida
30. V. Dasaratham
31. Manmohan Meena
32. Srihari Ram
33. Mohd. Maqbool
34. P. Shiva Shanker
35. S. Hanumantha Rao
36. K. Chandran
37. Mirza Ghouse Baig
38. G. Yadagiri
39. Mohd. Yaseen
40. K. Balraj
41. R. Narasimhulu
42. Mohd. Chand
43. Mohd. Maulana
44. Shaik Mukthar
45. K. Kasipathi
46. M. Leela
47. Manchikanti Krishna
48. Syed Asif Ali
49. K. Gopal
50. B. Eswaraiah
51. Mohd. Issamaiah
52. K. Harinarayana
53. E. Mallesh
54. Mohd. Sabeer
55. K. Narender
56. Y. Ramulu
57. M. Somaiah
58. E. Ganesh
59. G. Venkatesh
60. V. Somaiah
61. E. Marthamma
62. T. Govind
63. P. Srihari
64. Mohd. Habibuddin
65. A. Venugopal
66. M. Kisan

Attendant (Services) Contd..
67. B. Nageswara rao
68. P. Nagulu
69. M. Seenu
70. B.K.Mahadevaiah
71. A. Chandraprakash
72. M. Narasimha
73. M. Jayamma
74. D. Venkaesh
75. M. Satyamma
76. C. Sivaleela
77. G. Satyapual
78. A. Narsing Rao
79. A. Lakshmi
80. Majeed Shareef
81. M. Upender
82. R. Punna Reddy
83. K. Srinu
84. M. Narsing Rao
85. A. Shanker
86. P. Ravinder
87. D. Madhava Reddy
88. B.V. Sudershan Babu
89. I. Posheety
90. G. Yadagiri
91. M. Venkataiah
92. N. Bhasker
93. A. Jangaiah
94. P. Dasarath
95. S. Narahari
96. K. Venkatesh
97. P. Narasimha
98. E. Kondal Reddy
99. K. Venkat Reddy
100. G. Upender
101. M. Koumura Reddy
102. Ch. Shanker
103. G. Saraswathi
104. P. Balrajun

RESEARCH HIGHLIGHTS

1. COMMUNITY STUDIES

1.1 Diet and nutritional status of rural population and prevalence of obesity, hypertension and diabetes among adults of > 18 years in India: NNMB Third repeat survey

The study was carried out in 10 major states of India during 2011-12. A total of 86,754 subjects were covered for carrying out nutritional anthropometry and clinical examination to assess nutritional deficiencies. Whereas, food and nutrient intakes, obesity, hypertension and diabetes were assessed on sub-sample from 23,889 households in 1195 villages. A marginal decline was observed in the intake of all the food stuffs except, pulses, green leafy vegetables and fats and oils over decades. Also, average intake of all the nutrients had declined in the same period. The overall prevalence of underweight among preschool children had declined from 76% in 1975-79 to 49% in 1996-97 and to 41% in 2011-12. The figures for decline in stunting during the same period were 82%, 53% and 46% respectively and for wasting it were 27%, 23% and 16% respectively. The prevalence of hypertension was 22% and 21%, while that of diabetes it was 8.2% and 6.8% among men and women respectively.

1.2 Assessment of effects of consumption of 'Carbonated water beverages' and soft drinks on health of adolescents and young adults

The major reasons for the increased prevalence of overweight and obesity especially among youngsters could be transition in socioeconomic status, faulty nutrition (eg. sugar sweetened beverages), unhealthy lifestyles and physical inactivity levels among the population. It places these children at risk during their early and latter life. Therefore, the above study was carried out to assess ill effects of consumption of carbonated beverages. A total 2,035 young adults (men: 48.6%) in the age group of 18-35 years were covered and similarly, a total 2,257 adolescents (boys: 56.5%) were covered for assessment. Adults, consuming CWBs $\geq$400 ml/week were defined as consumers and those who were not /occasionally consuming were defined as non–consumers. The mean consumption of CWBs per day was 65ml/day and 20ml/day among adult men and women respectively. The mean intake of CWBs was 47.3ml/day and 26.6ml/day among boys and girls respectively.

1.3 Preparing sites for conducting effectiveness trials of microbicides in India – Multicentric study conducted in Maharastra, Karnataka and Andhra Pradesh

The study was carried out on female sex workers in the districts of E.Godavari and Khammam as a part of the multicentric study in the State of Andhra Pradesh, to assess the needs for options of prevention of HIV transmission in females and their willingness to participate in phase III clinical trials with intervention of “microbicides”. The study covered 3000 female sex workers and majority of them were rural based, married and illiterate. The mean age at first sex was about 15 years. About 27-42% of the FSWs expressed their willingness to use microbicides. The prevalence of HIV was 15.9% and 10.4% in E.Godavari and Khammam districts respectively.

1.4 Nutrition profile of Chenchu – A primitive tribe of Andhra Pradesh

Chenchu is one of the PTG recognized by Government of India. Generally, their dietary habits, culture and their way of life are different from the general population. Therefore, the present study was carried out during 2012 to assess their diet and nutritional status. There were no differentials observed in their food and nutrient intakes when compared to their tribal counterparts of the State. However, the prevalence of underweight, stunting and wasting was lower as compared to the general tribes of Andhra Pradesh. As observed in the verbal autopsy, the major cause of deaths among infants was premature delivery and low birth weight and among adults, it was cirrhosis of liver and accidents.

1.5 Assessment of nutritional status of below five year rural children and performance of ICDS functionaries in the districts of Gujarat State

The Government of Gujarat plans to develop State and District Nutrition Policy and develop plan of action for its implementation in priority districts. In this connection, NIN carried out a survey and district wise mapping of undernutrition was done. A total of 12,929 children (Boys: 52.3%) of <5 year age old were covered from the selected 10,424 households in 520 Anganwadi Centers for the purpose.

Identification of geographical areas with similar patterns of undernutrition prevalence and factors contributing for undernutrition is essential to develop and implement region-specific intervention strategies.

For this purpose, cluster analysis was carried out to identify three geographical areas/groups of districts in the state, with extent of underweight (high, medium and low) among children of <5 years as criterion.

The overall prevalence of underweight was 59% in cluster 1(red map), 46% in cluster 2 (yellow map) and 33% in cluster 3 (green map). It was observed that the proportion of children belonging to SC/ST population, joint families, illiterate parents, households having agricultural land and major occupation of parents being labour was significantly ($p < 0.01$) higher in cluster 1.

2. CLINICAL STUDIES

➢2.1 Nutritional challenges, abdominal adiposity and type 2 diabetes in Indians Parental and offspring cardio-metabolic risk: A trans-generational extension of Hyderabad Nutrition Trial (Andhra Pradesh Children and Parents Study-APCAPS)

- ➢. A cohort study was done to assess the impact of ICDS food supplement given to pregnant women in 29 villages around Hyderabad (15 under ICDS program & 14 control) on the CVD risk factors in the offspring.
- ➢ Birth weights were higher in the ICDS covered pregnant women(1987-90)
- ➢ In 2003-2005, in the first follow-up, when the subjects were13-18 year old, supplemented group were taller and had more favourable measures of insulin sensitivity and arterial stiffness (1165 adolescents).
- ➢ Second follow-up in 2009 to11 showed no difference on body composition or cardio-metabolic risk in the supplemented versus unsupplemented rural young adults.
- ➢ Current lifestyle factors - diet, physical activity, vitamin D status were the major determinants of body composition and cardio-metabolic risk during the second follow-up.

3. MICROBIOLOGY AND IMMUNOLOGY

3.1 Anticarcinogenic property of probiotic in combination with Allium sativum and NSAIDs on DMH induced colon cancer and colon cancer stem cells in rats

- ➢ Treatment with Allium Sativum, Lactobacillus rhamnosus GG and NSAIDs prevented DMH induced histopathological alterations and tumour formation in the colonic tissue; and inhibited Wnt and β-catenin expression.
- ➢ Microarray experiment demonstrated that PI3-kinase/Akt and Wnt/β-catenin pathway mediates key signals for intestinal epithelial cell proliferation and inhibition of apoptosis in an experimental model of colon cancer.
- ➢ Inhibition of Wnt and β-catenin pathways and induction of GSK-3b, which may eventually activate apoptosis and block key signals for intestinal epithelial cell proliferation appear to be the potential molecular pathway.

3.2 Metabolic endotoxemia and associated metabolic disorders in rats fed different diets and their relation to selective gut bacteria and changes in intestinal permeability – Phase 1 and Phase 2

i. *Effect of various cooking oils :* Effect of 10% groundnut oil, sun flower oil, palm oil, ghee and hydrogenated fats in an isocaloric diet fed to Sprague-Dawley rats for 5 months was studied. The study shows adverse effects on body composition, insulin sensitivity, bone mineral density and altered gut bacteria (lower bifidobacterial count and higher bacteroidetes) in rats fed hydrogenated vegetable fat (oil) for 5 months. Nevertheless, endotoxin levels, ASmase and reactive oxygen species were normal suggesting intact intestinal integrity that signifies other mechanisms underlying the changes observed in body composition and insulin sensitivity.

ii. *Effects of feeding high carbohydrate diet:* In the rats fed high carbohydrate diet (starch at 78%), there were changes in body composition like increased body fat%, decreased lean body mass, decreased fat free mass and decreased HDL/cholesterol ratio, increased plasma triglycerides. Rats, which received higher percentage of starch had higher Firmicutes bacteria.

iii. *Effects of feeding high fat diet:* Palmolein at 30% induced systemic inflammation and altered gut bacteria profile unfavorably. Supplementing flax seed oil significantly ameliorated many of the high fat diet induced adverse effects and increased the beneficial bifidobacteria and reduced the firmicutes level. While high fat diet is found to influence immunity, systemic inflammation and gut bacteria unfavorably, high carbohydrate diet was found to have atherogenic effects like adversely affecting body composition and lipid profile. Flax oil had a prophylactic role in reversing many of the high fat diet induced changes.

4. BASIC STUDIES

4.1 Enhancing dietary iron and zinc bioavailability in Indian children

To achieve dietary adequacy of iron and zinc by using food-based approaches, food preparation and dietary practices must be considered. As iron and zinc in cereal-pulse based habitual diet is poor in its bioavailability, their deficiencies usually occur concurrently in the Indian population. Therefore, it is not possible to meet the recommended levels of iron and zinc through a food-based approach unless diversified with fruits rich in vitamin C or flesh foods. Iron and zinc absorption simultaneously from habitual rice-based meal diversified with 100 g of guava in 16 girls and 16 boys of 13-15 years using stable isotopes were assessed. The results demonstrated that dietary diversification with guava enhanced bioavailability of non-heme iron from 10% to 20% but not that of zinc. This can form simple inexpensive intervention strategy to combat iron deficiency anemia in India. However, there is a need to assess the impact of long term consumption of guava fruit in improving iron status among vulnerable segments of the population. Additionally this study provided absorption data for computing nutrient requirements and RDA of iron and zinc in adolescent boys and girls.

4.2 Isolation and characterization of an iron absorption enhancer from human milk.

Iron absorption from human milk reported to be high but the factors involved remained elusive since long time. In this study for the first time we demonstrated reduction and solubilization of ferric iron and its provided evidences for its association with increased iron absorption in intestinal cells. Further, we demonstrated complementation of intestinal ferric reductase (Dcytb) with low molecular weight human milk fractions. Heat denaturation of these fractions led to inhibition of ferric iron reducing activity but not the solubilization. However, addition of zinc or citrate lyase treatment led to inhibition of both ferric iron reduction, solubilization and intestinal cell uptake of iron. These findings demonstrate that citric acid present in human milk solubilizes the ferric iron which could be reduced by other heat labile components leading to increased iron absorption in intestinal cells.

4.3 Stress, allostatic load and micronutrient status: Impact of dietary advice

Adolescent age group is considered as susceptible to stress, micronutrient deficiencies and allostatic load (AL) and therefore, the relationship and the impact of nutrition education intervention on these three variables were tested. The one year prospective institution- based study was carried out in 370 adolescent boys (15-19y) from 5 schools of Greater Hyderabad Municipal Corporation. One third of the participants had allostatic load and the AL index showed a positive association with controllable life events of adolescent life event stress scale. Psychological stress was associated with elevated concentrations of inflammatory marker CRP and hepcidin which did not contribute to hypoferraemia, which may be due to the influence of various factors including multiple micronutrient deficiencies.

Among the micronutrients, vitamin B-12 was positively associated with adaptive coping which needed to be probed further. Even though there was an improvement in knowledge on micronutrients after intervention, only ascorbic acid and retinol status improved while vitamin B-12 and ferritin concentrations declined, but there was no reduction in allostatic load or stress. Taken together, these results suggest that high psychological stress lead to high allostatic load. Psychological stress was found to be pro-inflammatory leading to elevated hepcidin concentrations but not hypoferremia and therefore, the low-grade inflammation appeared to not interfere with iron absorption among adolescents.

4.4 Assessment and validation of body composition using different techniques and development of regression models in Indian population

Body composition studies are used in a wide variety of fields including human biology, medicine, epidemiology, human nutrition and sports science. The fat content of the human body has both physiological and medical importance. Thus, the measurement of the total body composition such as fat free mass (FFM) and fat mass (two compartmental model) provides useful information.

The information pertaining to assessment of body composition using various methods and available regression equations to predict body composition were developed on western population. However, the composition and the density of FFM in different individuals limit the accuracy of these measures and influenced mainly by age, gender and ethnicity.

Since, the Indian population are different in their size and stature as compared to the western population, the existing regression equations to predict body composition based on two compartmental model (skinfold thickness and bioelectrical impedance method) developed on western population may not be applicable in the Indian context and hence, the present study was carried out to validate these methods in our population to get an accurate measures of body composition. The project work on “Assessment and validation of body composition using different techniques and development of regression models in Indian population” has been completed. The results of the study would be potentially useful in three areas:

a. *Basic Research:* The data related to body composition in different age and gender bands is being generated for the first time in the Indian context based on the validation studies.

b. *Public Health Importance:* Accurate appraisal of body composition has greater public health significance keeping in view of the increased incidence of overweight and obesity and other NCD's in the country.

c. *Translational Research:* The regression models developed based on the skin fold technique, a cost effective tool, to predict body composition suitable to Indian Population will be translated into practical applications that will help various stakeholders like policy makers, clinicians, public health workers, nutritionists, weight management professionals, athletes etc. In addition, the results of the study would also facilitate in validating and redefining the cut-off-points for Body Mass Index (BMI) and Waist Hip Ratio (WHR) suitable to Indian population

based on the degree of association with fat mass as measured using density value obtained either by underwater weighing/air displacement techniques.

4.5 Amino acid – Metal complexes as model for the glucose tolerance factor of yeast: Hypoglycaemic activity and therapeutic potential in diabetes; Synthesis, structure and mechanism of action in yeast and animals

To validate/ negate the hypothesis that Binary/ ternary AA – Chromium complexes could be useful as insulin like/ hypoglycemic agents in the treatment of Type ½ Diabetes. The effects of synthetic Phe- Cr, Lys- Cr and Cys- Cr complexes on OGTT in five month old male SD rats (control and STZ induced diabetic) was evaluated. The salient findings are: i) Phe–Cr complex but not others showed a significant improvement in OGT (decreased AUC glucose) albeit in only diabetic rats but not age/ sex matched controls. ii) chronic oral supplementation with Cr: $(Phe)_3$ improved glucose tolerance in a high sucrose induced rat model of insulin resistance. iii) Modulation of insulin signaling but not altered secretion of insulin during OGTT appears to underlie Chromium Phe complex mediated alleviation of diet-induced insulin resistance. iv) The skeletal muscle from Cr (D-phe) 3-treated rats had enhanced Akt-phosphorylation and membrane translocation of GLUT4 compared to untreated diabetic controls appear to suggest that nutritional supplementation with chromium complexes may have potential therapeutic value in alleviating or preventing insulin resistance and the associated type-2 diabetes and metabolic syndrome.

4.6 IGF1and BDNF signaling in the brain of Wistar NIN obese mutant rats during ageing: Effect of calorie and micro-nutrient restrictions

To validate/ negate the hypothesis that modulation of brain IGF1/ BDNF signaling and/or greater oxidative stress underlie accelerated ageing in WNIN–Ob rats, studies were conducted in WNIN/Ob and corresponding control rats. The salient findings of these studies are : I) Altered IGF1 and BDNF levels and signaling in brain and / or high oxidative stress could underlie accelerated ageing in the obese mutant rats of NIN. ii) Significant changes were observed in plasma CRP levels in WNIN/Ob rats as compared to controls albeit at 12 months of age but not earlier. iii) Increased expression of glial fibrillary acidic protein (GFAP) in the hippocampus of 6 months old WNIN Ob rats compared to controls whereas, at 12 months of age GFAP expression was lower in WNIN/Ob than controls probably suggesting that it could underlie impaired synaptic plasticity and hence brain ageing in WNIN/Ob rats. iv) At 12 months of age WNIN/Ob rats showed higher ($p<0.001$) expression of ORX-A and ORX-A positive neurons in the brain than controls. Considering the importance of orexin in energy balance, feeding, wake–sleep cycle, stress response, aging, reproduction etc, the increased ORX-A expression could be another factor underlying accelerated ageing reported earlier in WNIN/Ob rats.

4.7 Functional assessment of adult human pancreatic islets following autologus transplantation

The study used these immunoisolatory devices (Theracytes) to test the viability and functionality of monkey islets, in this devices following auto-or allo-genic transplantation in monkeys. The animal experiments were performed at National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad which has an approved primate animal facility.Islets remain viable and functional for 12 months in pancreatectomized nonhuman primates in both autologous and allogenic islet transplantation. Allo transplanted islets were viable and functional in the absence of an immunosuppression.

Poly tetra fluoro ethylene devices (Theracyte) has engraftment potential to sustain islet with functional /insulin secreting responses. Vascularization was more extensive in the neck region and explants obtained from interscapular region denoted it to be relatively a better site for implantation as compared to the thigh.

The promises of transplantation without the immunosuppressive drugs towards the maintenance of Glycemic status achieved by bio-compatible devices (theracyte membrane) in higher model system such as Non-human primates. While the obtained results are indeed encouraging, further studies are required in this regard to evaluate the curative potential of macroencapsulated islets using diabetic models.

4.8 Establishment of propagable cell lines from pancreas and adipose tissue of embryo and adult WNIN obese rats (WNIN/Ob & WNIN/GR-Ob)

WNIN Mutants rats (WNIN/GR-Ob and WNIN/Ob) were used as the model to study the adipose and pancreatic stress/inflammation as a phased gene expression studies, markers of stem cells, and their lineage commitment to form adipocytes or islets using primary cultures stromal stem cells /ductal epithelial cells.

Data showed an alterations in tissue milieu (to adapt to physiological shifts happening in conditions of obesity and metabolic syndrome (MS) both during early and prolonged phase of obesity/insulin resistance (IR) have been documented, though mechanisms leading to such state have remained elusive so far. Participation of several confounding factors that collectively co-precipitates for a state of profound inflammation in target tissues being appreciable in Mutants> lean > Controls and gets worsened as the animals age were showed.

These factors include, hypertrophy, macrophage infiltration (CD11b/TNF / IL6), apoptosis, adipose/ -cell vacuolation, hyperinsulinemia (HI), stress markers (RL-77/HSP104/TBARS) all of which correlated well with indices for Obesity (2-3 fold), IR (1.5-3fold) and HI (2-3 fold). Further, supportive data was also obtained from *in vitro* studies using stromal cells /islet cell cultures amongst phenotypes. Complementary data obtained from BM-MSCs also supported for the state of disease memory in Mutants as compared to Lean and Control.

Studies both *in vitro* and *in vivo* amongst phenotypes does advocate for the alteration in stem cell milieu during the state of meatobolic lesions depicted in mutants with obesity/IR /IGT/ HI, associated with pathophysiological conditions (adipose tissue, pancreatic, BM-MSCs), portraying features of pre-diabetic/T2D as compared of human scenario.

4.9 Characterization of active principles and mechanism of action of dietary aldose reductase inhibitors and antiglycating agents: (v) Isolation and characterization of -glucogallin as a novel aldose reductase inhibitors from *Emblica officinalis*.

Aldose reductase (ALR2) is a major target for the development of therapies to treat diabetic complications. The bioassay-guided isolation and structure elucidation of 1-O-galloyl-b-D-glucose β-glucogallin, a major component from the fruit of the gooseberry that displays selective as well as relatively potent inhibition of ALR2 *in vitro* was described. Further, β-glucogallin effectively inhibits sorbitol accumulation under hyperglycemic conditions in an *ex-vivo* organ culture model of lenses excised from transgenic mice over-expressing human ALR2 in the lens. This study demonstrates that, molecules from natural products such as β-glucogallin as therapeutic leads in the development of novel therapies to treat diabetic complications.

4.10 Impact of agents with potential use in functional foods on biomarkers for induction of age related diseases

Accumulation of intracellular sorbitol due to increased aldose reductase (ALR2) has been implicated in the development of diabetic complications. The inhibition of ALR2 by ellagic acid (EA) a bioflavonoid present in many dietary sources was described. EA inhibited ALR2 with an IC_{50} of 46 nM in a non-competitive manner. Further, EA is relatively more specific towards ALR2 over other member of aldo-keto reductase family. Molecular docking studies substantiate these findings. Further, EA suppressed sorbitol accumulation in human erythrocytes, rat lens and rat retina under high glucose conditions. Finally, significance of EA

was demonstrated in terms of prevention of loss of lens transparency under high glucose conditions in *ex vivo* conditions. Together, these observations suggest that EA holds a therapeutic promise to prevent or treat complications of diabetes.

4.11 Potential role of dietary nutrients vitamin A and polyunsaturated fatty acids (PUFA) on regulation of development and /or control of obesity using a genetic obese mutant rat model (WNIN/GR-Ob)–Nutrient-Gene Interaction

Chronic feeding of vitamin A-enriched diet (129mg per kg diet) to glucose-intolerant obese rats of WNIN/GR-Ob strain improved the hyperglycemia, glucose tolerance and muscle insulin sensitivity. Further, qRT-PCR data suggest the transcriptional regulation of the various important lipogenic pathway and adipokine genes of visceral adipose tissue and it was well corroborated with the increased adiposity and insulin sensitivity observed in these obese rats. Importantly, it appears that vitamin A-mediated improvement in muscle insulin sensitivity in obese rats is due to regulation of phosphorylation status of glycogen synthase, which in turn resulted in increased glucose uptake and glycogen synthesis.

4.12 Abdominal obesity and its relation to plasma homocysteine and other biochemical CHD risk factors in middle aged men

With a highest (58%) prevalence of hypertension in men with abdominal obesity measured by waist circumference (WC) ≥90cm and with a significant ($p<0.001$) positive correlation of the four obesity measures (BMI, WC, WHR and % of BF) with systolic and diastolic blood pressures, regression analysis shows that WC alone predicts systolic and diastolic blood pressure in the present study men.

With a 25 % prevalence of insulin resistance, it was observed that, a graded increase in insulin resistance from lower ≥18.5-<23), to medium ≥23.5-<27.5 and to higher range of BMI≥27.5 in the present study men. Regression analysis shows that BMI predicts insulin resistance in the present study men. Regression analysis shows that the inflammatory marker, C-reactive protein levels were found to be positively associated to waist circumference and % of body fat.

With a 28% prevalence of hyperhomocysteinemia (≥15µmoles/L) in the overall study population, vegetarian men had doubled the prevalence of hyperhomocysteinemia compared to non-vegetarian men.

In relation to body composition, in a similar range of BMI, men with high WC had significantly ($p<0.05$) higher plasma homocysteine concentrations than the men with normal WC (<90cm). With a 50% prevalence of low HDL-cholesterol (<35mg/dl) levels in the present study, men with low HDL_cholesterol levels were found to have significantly ($p<0.05$) higher plasma homocysteine concentrations than men with normal HDL-cholesterol levels. A negative correlation observed between plasma homocysteine and HDL cholesterol levels is an important finding of the present study.

5. EXTENSION & TRAINING DIVISION

5.1 Evaluation of nutrition reports based on research studies in leading Indian newspapers

Six newspapers including English and Telugu dailies were analysed to study the pattern of their coverage of nutrition related news/ features. It was found that about 70% of the items reported were based on secondary sources and only 30% were quoted from original source, out of which, very few based their reports on peer reviewed journals. Some of them carried the reports of public relations departments of food/ pharma industries. Emphasis was placed on 'newsworthy' pieces of information, especially those which run contrary to current health recommendations. Sample characteristics, study methodology, and study limitations are not routinely reported in the newspapers. The study further stresses the need for synergetic efforts between journalists covering health topics and experts in the field of diet and nutrition in order to avoid inaccurate information to the readers.

6. FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

6.1 Creation of demand for millet foods through PCS value chain

Sorghum has high levels of both soluble and insoluble fibre. Fibre is known to slow the release of glucose and has beneficial effects in diabetics who have exhibit high increase in post prandial plasma glucose levels. The newly developed cultivars have higher levels of protein. A project was initiated to create demand for millet foods through PCS value chain with a view to commercialize several products of sorghum. The studies revealed that products prepared from sorghum were organoleptically on par with similar recipes prepared from wheat or rice. They were acceptable to children also. Consumption of sorghum resulted in better control of hyperglycemia. Further, the glycemic index of some of the products were lower than recipes prepared from other cereals.

6.2 Value chain on commercialization of maize products

The human consumption of maize is less than 10% of its produce. National Agricultural Innovative Project, a division of ICAR has initiated project to develop innovative products of maize for commercialization. Two varieties of maize were used to prepare selected food items and evaluated for acceptability and management of blood glucose in Type 2 diabetics. The studies indicated that QPM variety of maize had better quality of protein than Nityashree. Regular consumption of maize based foods had significantly lowering effect on levels of glycosylated hemoglobin although they were not low in glycemic index and load. The resulting beneficial effect could be due to the fibre content in the grain.

6.3 Micronutrient profile of population residing in fluoride endemic areas

Fluorosis is caused due to chronic exposure to high fluoride through water and food. The condition is aggravated by malnutrition. Water in endemic fluoride areas have been reported to have low levels of some minerals like Ca, Mg and Cu. Micronutrient deficiencies have been implicated in the etiopathogenesis of fluorosis. A study was conducted to understand the role of micronutrients in fluorosis.

The levels of magnesium, selenium and zinc were significantly higher in individuals from areas endemic for fluorosis. Thyroid function test showed that levels of T3 was significantly higher in people belonging to areas where fluorosis exists. There were no changes in hematological parameters. Overall, the survey demonstrated that adverse effect due to exposure of high fluoride through water was less in the population studied due to their good nutritional status.

6.4 Assessment of pesticide exposure and various cancers among agricultural farming community Guntur District

Most of the farmers in Guntur district use organophosphote and synthetic pyrethroid pesticides to control pests on chilly and cotton. Incidence of certain types of cancers have been reported from the farming community. Literature evidence supports association of lymphoma, leukemia and breast cancers with exposure to pesticides. Therefore, a project was taken up to understand the role of farming practices, exposure to pesticides and cancer incidence. The results revealed that 89 subjects out of 670 cancer subjects showed presence of P, P'DDE and four of them had high levels. The survey also indicated that most of the farmers were not practicing proper spraying methods or adopting precautionary measures prior to/immediate after spraying.

6.5 Assessment of dietary intakes of select chemical and processing induced contaminants in various socio economic groups in Hyderabad

Levels of an exact intake of nutrients and exposure to contaminants through diet hitherto has been reported based on calculations done using raw food components. However, the actual exposure can be assessed only by calculating the intakes and exposure based on foods following processing/cooking processes.

The National Nutrition Monitoring Bureau (NNMB) provides information on food consumption of non processed foods and it is also rural based survey. In the present study, information on foods consumed (conventional and processed) by high income, middle income and low income was collected. Foods were cooked as is done in culinary practice.

The data showed that the intakes of conventional and processed foods differed between different socioeconomic sections among the Hyderabad population. Intake of trans fat through selected bakery items and other sources were less than 1% of energy. Dietary exposure to lead and cadmium were below the respective provisional tolerable weekly intakes (PTWIs). Estimated dietary exposure to pesticides were within the acceptable daily intakes.

6.6 Evaluation of herbal and nutraceutical product for anti-atherosclerotic activity

Earlier research findings have demonstrated anti-atherosclerotic activity (in-silico, *in-vitro* & *in-vivo*) of Poly Herbal Nutraceutical Formulation (PHN). The current studies suggest that prevention of foam cell formation due to possibly attenuation of CD36 cascading pathway, which promote entrapment of lipid laden foam cells due to ROS, inflammation and oxidized LDL (oxLDL) activation.

7. NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

7.1 Establishment of baseline values of body composition and blood pressure in different species of laboratory animals maintained at NCLAS – A Study on mice strains

Body composition analysis reveals the nutritional status and general well being of an individual. In the past, body composition of experimental animals was determined by carcass chemical method, necessitating the sacrifice of animals. But, in recent times alternative non-invasive measures like total body electrical conductivity (TOBEC) and dual X-ray absorbtiometry (DXA) have emerged, which allow repeated individual measurements without sacrifice of animals.

Progressive changes in the body composition of three commonly used mice strains in nutritional and toxicological research VIZ., Swiss albino, BALB/C and $C_{57}BL_6J$ were analyzed by TOBEC initially and subsequently by DXA compared with chemical method and parameters like lean body mass (LBM), fat, fat %, fat free mass were determined. It was observed that the TOBEC analysis did not correlated with the carcass analysis for mice.

DXA is superior, constant and correlated with chemical method and found to be more appropriate. Swiss albino mice had significantly higher body weights compared to BALB/C and $C_{57}BL_6J$. This was more pronounced in males than females. LBM is reduced, fat and total fat percentage was found to be more in Swiss albino than the other two strains. Similar pattern of results were obtained in terms of their food intake and clinical chemistry parameters.

In conclusion, the findings are unequivocally showed that for body composition analysis of lab animals like mice, hamsters, guinea pigs and rabbits, DXA is superior and constant in comparison with all methods, for rats, TOBEC could match that carcass analysis.

8. PRE-CLINICAL TOXICOLOGY

8.1 Pre-clinical efficacy (IBD, Anti-inflammatory) and safety evaluation of Novel Peptide Genopep 4 (Issar 4)

Inflammatory bowel disease (IBD), a major health problem in the developed world which comprises i) ulcerative colitis (UC) and (ii) Crohn's disease (CD) a chronic relapsing and remitting inflammatory disorders of the gastrointestinal tract. The Centre has evaluated a beneficial role through immune regulatory activity and anti inflammatory properties of IS-217 having similarities like interleukin10 (IL-10).

8.2 Pre-clinical toxicology study of Recombinant Interferon beta-1b (IFN 1b)

A private organization had developed Interferon beta –1b using recombinant DNA technology with an intention to promote it for the treatment of multiple sclerosis. The Pre-clinical toxicity investigation (Acute and sub–chronic) has been conducted as per DBT/Schedule Y of DCGI guidelines and report submitted for clinical trials through RCGM and DCGI.

8.3 Pre-clinical toxicity evaluation of Transgenic Cotton (Cry1ac Event-1and Cry1ecevent-24)

The Bt cotton with stack genes Cry1Ac (event-1) and Cry1EC (event-24) have been developed by one of the private companies It was evaluated for pre-clinical toxicology as a part of Bio-safety norms of DBT.

I COMMUNITY STUDIES

1 DIET AND NUTRITIONAL STATUS OF RURAL POPULATION AND PREVALENCE OF OBESITY, HYPERTENSION AND DIABETES AMONG ADULTS OF ≥18 YEARS IN INDIA: NNMB THIRD REPEAT RURAL SURVEYS

As per the recommendations of the NNMB Steering Committee, 3rd Repeat rural surveys were carried out during 2010-12 in all the 10 NNMB States to assess the diet and nutritional status of rural population and to study time trends in diet and nutritional status of rural population in comparison with the findings of the NNMB surveys carried out earlier during 1975-79, 1988-90 and 1996-97.

In view of steady increase in the prevalence of diet related chronic diseases like overweight and obesity, hypertension, diabetes mellitus even in rural population, relevant data pertaining to these problems were also collected in addition to information on diet and nutrition in all the states.

OBJECTIVES

To assess the current diet and nutritional status of rural population and time trends in all the 10 NNMB States and to assess the prevalence of overweight and obesity, hypertension and diabetes mellitus among adults of 18 years of age and above.

1. To assess the current status of food and nutrient intake among different age/sex/physiological groups of rural population living in 10 NNMB States.
2. To assess the current nutritional status of all the available individuals in terms of anthropometry and clinical examination for nutritional deficiency signs.
3. To assess morbidity during previous fortnight among all the individuals covered for anthropometry.
4. To study the time trends in the food and nutrient intakes and nutritional status since 1975-79, 1988-90 and 1996-97.
5. To assess the prevalence of overweight/obesity, hypertension and Type 2 diabetes among the rural adult men and women (>18 years) and their awareness about hypertension and diabetes.
6. To collect dried blood spots for subsequent DNA extraction and creation of a DNA bio-repository for future genetic epidemiological studies in collaboration with South East Asia Network chronic diseases (SANCD)/Public Health Foundation of India (PHFI).

METHODOLOGY

Study design

It was a cross-sectional, community-based survey carried out in 10 NNMB states in India, by adopting random sampling procedure.

Selection of villages

A total of 120 villages were covered in each NNMB State. Of these, 90 villages were selected from those that were covered in baseline (1975-79), first repeat (1988-90) and second repeat (1996-97) surveys and the remaining 30 villages were covered, which were randomly selected afresh from the list of villages obtained from the census 2001.

SAMPLE SIZE ESTIMATION

Nutritional status

All the available subjects from the selected 2400HHs were covered for anthropometry, clinical examination and history of morbidity. A 24 hour recall method of diet survey was carried out in every alternate HH, covered for nutrition assessment in each village. The sample size required for each state for various investigations among different target groups of individuals are given in table 1.

Table 1

Investigations	No. of HHs	Age/Sex/ Physiological Group	Prevalence	C.I	Relative Precision	Sample required per State
Anthropometry	2400	All the available individuals				
Clinical Examination						
History of morbidity						
Diet survey	1200	All the individuals partaking meals in the HH				
Blood Pressure measurement	2400	Men (18yrs)	15%	95%	20%	1100
		Women (18 yrs)				1100
Measurement of fasting blood sugar	2400	Men (18 yrs)	10%	95%	20%	1730
		Women (18 yrs)				1730
Knowledge & Practice on HTN & DM	2400	Men (18 yrs)	-	-	-	1730
	2400	Women (18 yrs)	-	-	-	1730

Sampling procedures and selection of subjects

In each of the selected villages, 20 HHs were covered randomly. For the purpose, each village tentatively divided into 5 geographical areas based on the lanes/by lanes/streets and 5 the was always SC/ST colony if exists. In each geographical area, 1st HH was selected by following random procedure and consecutively another 3 HHs were also covered. Thus, 20 HHs were covered, 4 each from all the 5 geographical areas. All the available individuals from each of the selected HH were covered for various proposed investigations. While collecting data, written individual consent was taken.

The salient findings of the study are as follows:

- A total of 86898 individuals were covered for nutritional anthropometry, clinical examination and current history of morbidity, from 23889 households in 1195 villages of the 10 States.
- Data on food and nutrient intake was collected from 50,251 individuals of different age and sex groups from about 23889 households.
- Measurement of blood pressure, waist and hip circumference and information on knowledge and practices about hypertension and diabetes was also collected among 49,323 adults of 18 years, and
- Fasting blood glucose levels was also carried out in 32,831 adults ($\geq$18 yrs).
- Infant and young child feeding practices were also assessed among 4,459 mothers of <3 years children.

NUTRITIONAL STATUS

Food and Nutrient intake

- The mean intakes of most of the foodstuffs were below the RDI. Even though, the consumption of protective foods, such as green leafy vegetables, milk and milk products, fruits, sugar and

jaggery has increased marginally, the consumption levels were grossly deficient when compared to recommended levels.

- The intakes of all the nutrients in most of the states, were below the recommended levels, particularly of those micronutrients such as vitamin A, iron and riboflavin.
- Among the pregnant and lactating women, the median intakes of all the nutrients were below the RDA. The extent of deficit was relatively more for vitamin A, iron, calcium and folic acid among children and pregnant women.
- Only about 50-60% of the preschool and 40-55% of school age children had adequate intakes of both protein and calories. About 63% of adult men and women had adequate intakes of both protein and calories.
- Among the adult women, protein-calorie adequacy was found to be more among NPNL women (71%) as compared to pregnant (52%) and lactating women (60%).

Anthropometric Indices

- The overall prevalence of undernutrition (<Median – 2SD) among infants in terms of underweight, stunting and wasting was 27%, 23% and 25%, respectively and it tended to increasing with increase of age.
- The prevalence of undernutrition was higher among preschool children (45%, 48% and 21% respectively), except wasting as compared to infants.
- It was observed that the prevalence was high in the States of Madhya Pradesh, Maharashtra, Gujarat, Uttar Pradesh and Orissa.
- The prevalence of undernutrition among school age and adolescents (<5 centile of BMI: thinness) was high in 6-9 years: (36.5% & 31%); 10-13 years (47% & 36%); & 14-17 years (41% & 23%) among boys and girls respectively).
- The prevalence of chronic energy deficiency (CED) was 35% each among adult men and women respectively.
- The prevalence of CED was higher among men in Uttar Pradesh and Gujarat (46% & 43% respectively while among women, it was higher in Orissa, Gujarat & Uttar Pradesh.

Time trends in diet and nutritional status (date available for 7 States)

- There was a marginal decline in the intake of most of the foods except pulses, GLV and fats & oils at HHs level. The average intake of all nutrients except thiamine has declined over the periods from 4 decades.
- A significant reduction in the nutritional deficiency signs such as kwashiorkor, marasmus, vitamin A and B-complex deficiencies among preschool children was observed over the period.
- The overall prevalence of underweight among 1-5 year children has declined from 49% to 41%, stunting has declined from 53% to 46%, while that of wasting has declined from 22.5% to 15.5% since last 3 to 4 decades.
- The prevalence of chronic energy deficiency (BMI<18.5) (adult nutritional status) decreased from 45.5% to 32% among all adult men and from 48% to 33% among adult women during 1997-98 and 2010-11.
- Marginal improvement in the overall nutritional status observed over a period of time despite of decline in nutrient intakes. The improvement in nutritional status could be due to non-nutritional factors, such as improved accessibility to health care facilities, sanitation, protected water supply, and increased purchasing power, Govt. Scheme such as TPDS, PDS etc.

Diet related chronic non-communicable diseases

The prevalence of overweight and obesity (BMI ≥25) among rural men and women was 10% and 13.5% respectively according to global criteria.

The prevalence of hypertension (SBP 140 mm of Hg and /or DBP 90 mm of Hg) was about 19% among men, and 18% among women according to JNC 7 criteria. The prevalence was highest in the State of West Bengal (men: 28%; women: 27%) and lowest in Madhya Pradesh (men: 13% & women 16%) and Uttar Pradesh (17% & 14% respectively) and Andhra Pradesh (14% & 12% respectively). About 81% of men and 72% of women were aware of hypertension. Only 5% men & 6% women were known hypertensives and only about 5% were on treatment.

The prevalence of diabetes was 6-7% among adult men and women of ≥18 years and 8-9% adults were also suffering from pre-diabetes stage (impaired glucose tolerance). About 75% men and 67% women were aware of diabetes mellitus. Of them about 3% each were known diabetics. About 51% of men and 17% of women were smoking tobacco in various forms, the proportion of which was higher among 50-70 years compared to 18-30 years age group. About 8-14% was chewing tobacco, while about 5-15% was snuffing the same.

The consumption of alcohol was higher among men (28%) compared to women (4%). Daily consumption of alcohol was reported by about 4% of adult men.

The study revealed that a significant decline observed in the prevalence of undernutrition over a period 4 decades. However, still it is an important public health problem when compared to the figures of developed countries. Therefore, there is an urgent to strengthen all the existing national nutrition intervention and other support programmes focussed towards food and nutrient security. The prevalence of hypertension and diabetes was also significantly higher among rural adult population, especially in the states of Kerala, Maharashtra, Odisha and West Bengal and it was also observed that the awareness levels were very low. The prevalence of smoking of tobacco and consumption of alcohol was very high among rural men which are identified as important risk factor for hypertension and diabetes.

Therefore, there is a need to increase awareness levels on obesity, hypertension and diabetes, including its future consequences through IEC and BCC techniques.

2 ASSESSMENT OF EFFECTS OF CONSUMPTION OF CARBONATED WATER BEVERAGES ON HEALTH OF ADOLESCENTS AND YOUNG ADULTS

The prevalence of overweight and obesity among urban children, adolescents and young adults is increasing in epidemic proportions in developed as well as in developing countries, including India. Overweight and obesity place these children at increased risk of significant health problems, both during their early life and during adult life. Several Indian studies also revealed that the prevalence of overweight and obesity was 10-15% among school age and adolescent population. The major reasons could be transition in nutrition, socioeconomic and demographic factors, life styles and physical inactivity levels.

A recent review had indicated that greater consumption of sugar-sweetened beverages is associated with weight gain and obesity. The carbonated beverages (300 ml/day), apart from

making a significant contribution to sugar intake (15-20g) also contains 60mg of Caffeine (permitted level in India). In a meta-analysis of 88 studies, found the association between carbonated beverages and soft drink consumption and nutrition and ill effects on health. It was clearly stated that soft drink intake increased the body weight and it was also associated with lower intakes of milk, calcium and other nutrients.

Since there is a paucity of the data on carbonated beverage consumption and its ill-effects on health of adolescents and adults in India, the present study was carried out with the following hypothesis and objectives:

METHODS

Since there is a scarcity of the data related to consumption of carbonated beverages and its effect on body weight, BMD, mental distress and hypertension on adolescents and young adults in Indian context, the present study was carried out to test the following hypothesis:

Hypothesis

- Consumption of CWBs have adverse effects on body weight, bone mass and bone mineral density (BMD) in adolescents and young adults, and
- Regular consumption of CWGs causes hyperactivity, mental distress and conduct problems
- among adolescents and young adults..

OBJECTIVES

Primary objective

To assess the consumption levels of carbonated water beverages (CWB) in adolescents and young adults and its effects on body weight, bone mineral density, mental distress and hyperactivity.

Secondary objectives

1. To assess the profile and determinants of consumption of carbonated beverages (CWBs) among urban adolescents and young adults,
2. To assess, over a one year period, the adverse effects of regular consumption of CWBs on anthropometric indices of growth, bone mineral density, mental distress, and hyperactivity.
3. To assess the levels of sugar, phosphoric acid and caffeine in CWBs in various brands available in the market.

METHODS

Phase I study was cross sectional with a two stage cluster sampling design and phase II was a longitudinal study, in which consumers and non-consumers of CWBs were followed up for one year to determine the association of consumption of CWBs with anthropometric, biochemical parameters, body composition (DEXA) and bone health, psychological wellbeing and lifestyles.

Sample Size

- Assuming that 10% of adolescents (10-15 years) or young adults aged 18-35 years are regular consumers of CWBs, the required study sample size was estimated to be 2000 for obtaining an estimate with relative precision of 20% and 95% confidence level, with a design effect of 2 and dropout rate of 10%.
- Assuming that the mean BMD in non-consumers is 0.9 with S.D of 0.15, and that the expected difference between non-consumers and consumers is 10%, i.e., 0.09, the required sample size for each group is 85, with 95% power and with a significance level of 0.01.

- About 200 consumers and non-consumers were assessed for their mental distress and hyperactivity by using appropriate scales. This part of the study was carried out after identification of consumers and non-consumers.

Selection of subjects

From a total of 700 schools, 16 schools were selected randomly and 30 municipal wards were selected. From each selected school, about 120 adolescents (60 boys and 60 girls) were covered for the present survey. From each cluster, 35 men and women each from middle and high socioeconomic groups were selected with a random start. The first adult was selected randomly from North East corner of the selected ward and rest of them were recruited contiguously, until the required number were covered.

Selection of consumers and non-consumers

To select adult consumers, 2,035 adults were interviewed with the help of piloted study instruments and information was obtained on various parameters, including consumption levels of CWBs. Those adults who were consuming CWBs ≥400ml/week were categorized as consumers. Accordingly, out of 2,035 adults surveyed at baseline, 199 (men: 154; women: 45) were identified as consumers. Out of 2,257 adolescents screened, those who were consuming CWBs ≥600ml/week were selected as consumers (304).

All those who were not consuming CWBs at all and those adolescents who were consuming occasionally were categorized as non-consumers (223 and 317).

Data pertaining to socioeconomic and demographic particulars, anthropometry, knowledge, practices and lifestyle factors, 24 hr recall method of diet surveys, FFQs, DEXA, estimation of CWBs constituents, effects of CWBs on hyperactivity and mental distress were also assessed.

Ethical clearance was obtained from Institutional Ethical Review Board (IRB), National Institute of Nutrition, ICMR, Hyderabad and written informed consent was obtained from the Heads of the institutions and assent from the adolescents. Written informed consent from the parents of the children, whose blood samples, DEXA and psychological assessment was carried out. In case of adults, written individual informed consent was obtained.

The following are salient findings of the study:

- A total 2,035 young adults (men: 988; women: 1047) in the age group of 18-35 years were covered from the randomly selected 30 Municipal wards of Greater Hyderabad Municipal Corporation (GHMC).
- Similarly, a total 2,257 adolescents (boys: 1276; girls: 981) were covered from 16 randomly selected private schools, Hyderabad, Andhra Pradesh.
- More than half (57.2%) of men and one fourth (27.7%) of women were consuming CWBs at least once in a week. A significantly higher proportion of men (79.5%) were consuming CWBs at least once in a month compared to women (59.2%). Among those who consumed, half of the adults (52.2%) were consuming CWBs for more than 10 years. Only 8% of adults were consuming CWBs more than 1200ml/week. With respect to adolescents, a higher proportion of boys (82%) were consuming CWBs compared to girls (70%) after excluding occasional consumers.
- Only 5% of adolescents were never drinking CWBs. Only 7% adolescents' were consuming for more than 10 years, while half of boys and one third of girls were consuming since more than 7 years. The reasons reported for CWB consumption by the study participants were its taste or helps in thirsty/digestion, time pass, peer pressure, addiction to CWB consumption, influence of TV advertisements, high prestige value, its ready availability and convenience.

- At the screening phase, it is observed that women consuming ≥1200ml/week were having relatively higher prevalence of overweight, abdominal obesity and percent body fat compared to the women consuming 600-1200ml/week. While in men, the prevalence of overweight and obesity and percent body fat was significantly higher ($p<0.043$) among men who were consuming >10 years compared to 6 years or less.
- The prevalence of hypertension was higher among men of 20-35 years (17.1%) compared to women (8.3%), who were consuming carbonated water beverages (CWBs) ≥1200ml/week as compared to<600ml/week (14.7%) and (2.8%) respectively.
- The prevalence of obesity and overweight was more or less similar at various levels of consumption among adolescents and non of adolescents were suffering from hypertension.
- The consumption of cereals and millets among adult consumers was 274g/day, while among non consumers; it was 262g/day as against RDI of 460g/day. Similarly, intake of all the nutrients among men was below the RDI. Incase of adolescents, the mean intakes of milk and milk products was higher in non-consumers of CWBs compared to consumers, which reflected in higher intakes of calcium among non-consumers.
- At baseline, no significant differences were observed between adult consumers and non consumers of CWBs with respect to all the anthropometric, biochemical, parameter of body composition (DEXA), obesity and HTN. Similar findings were observed in midterm (Round I) and end line surveys (Round II) with respect to the above parameters. However, increments from baseline to end line, with respect to BMI ($p=0.015$), percent body fat $p=0.001$), weight ($p=0.004$) and WC ($p=0.046$) were significantly higher among the consumers.
- However, mean increments with respect to DEXA and biochemical parameters were not significant. Similarly, no significant differences were observed in adolescents, between consumers and non-consumers of CWBs with respect to anthropometric, biochemical and, bone parameters at baseline. Similar observations were found during midterm and end line surveys.
- The mean increments with respect to body composition (DEXA) biochemical parameters and BMI were not significantly different between the consumers and non-consumers of CWB. On the psychological well being parameters, there were no significant differences between both the groups in terms of anxiety, depression and Attention Deficit Hyperactivity Disorder (ADHD) among adults.
- However, significantly higher scores in conduct problems, emotional symptoms and trait anxiety were observed in adolescent consumers compared to non-consumers. After controlling for life events, there were no significant group differences but only a trend was observed.

CONCLUSIONS

- The present study showed significantly higher increments in weight ($p=0.004$), percent body fat percentage ($p=0.001$), WC ($p=0.046$) and BMI ($p=0.015$) among CWB consumers compared to non-consumer adults.
- No similar differences were found in case of adolescents. In adolescents, significantly higher scores were observed in conduct problems, emotional symptoms and trait anxiety among CWB consumers. However, when controlled for life events, only a trend was observed.

RECOMMENDATIONS

- Promotion of carbonated water beverage consumption should be discouraged.
- As a primary preventive measure, the availability and sale of CWBs should be minimized in the premises of educational institutions.

- Consumption of healthy beverages such as fresh fruit juice/ low fat milk/coconut water/butter milk should be encouraged.
- Industries should be encouraged to produce and sale of low cost fresh fruit juice/ low fat milk/coconut water/butter milk etc.

Therefore, to achieve above strategic recommendations, cooperation and coordination is required from all the stakeholders such as governments, educational institutions, NGOs and parents.

3 PREPARING SITES FOR CONDUCTING EFFECTIVENESS TRIALS OF MICROBICIDES IN INDIA. II – MULTICENTRIC STUDY CONDUCTED IN MAHARASTRA, KARNATAKA AND ANDHRA PRADESH

Studies conducted earlier revealed that condoms play an important role in the prevention and control of HIV and STI among FSWs and their clientele. But ensuring the consistent use of condoms needs compliance from their male partners, also using female condoms by FSWs as an alternative is very expensive, therefore, there is need to develop female-controlled, alternative and reliable methods for effective control and prevention. The vaginal microbicides could prove to be an effective answer to the problem, therefore the current study had been undertaken with the aim to conduct phase III clinical trials with "microbicides".

The objectives, rationale, hypothesis, methodology and the outcome of the study were presented in the annual report of 2009, 2010 and 2011.

The objective of this study was to prepare sites for conducting Phase III clinical trials of HIV prevention technologies in India, in terms of selection of high HIV incidence and prevalent districts, assessing the needs of the population for female controlled options of prevention of HIV and assessing their willingness to participate in future HIV prevention trials.

The stage I of the study comprising assessment of current prevalence and incidence of HIV among high risk women "FSWs " in 3 states (2 districts each) with high HIV prevalence as per NACO sentinel surveillance data is being carried out in the states of Andhra Pradesh, Karnataka and Maharashtra. The sites with HIV incidence >3 per 100 person years among women will be considered for conducting an effectiveness trial with a candidate vaginal microbicide, as stage II of the study. In the state of Andhra Pradesh, the study is being carried out in two districts, E.Godavari and Khammam.

Salient features of the study

- A total of fifteen hundred subjects were recruited for each of the study district.
- The mean age of the FSWs was about 30 – 31 years and they were mostly ruralbased and married.
- Mean age at initiation of commercial sex was about 25 years and they were involved in commercial sex for a mean duration of 3.5 - 6 years.
- The mean number of clients per month varied between 5.6 -10.5, and they were mostly regular clients.

- A majority of the FSWs had multiple sex partners such as husband, friends, regular clients and occasional clients etc.
- About one fifth of them were also consuming alcohol.
- A majority of them insisted on the usage of male condom for contraception, but a small proportion of them were also using female condom.
- Most of the FSWs interviewed were aware of male condom and were willing to obtain them from free clinics.
- About 38 - 45% of the FSWs were using condom every time they had sex, however about 3-5 % of the subjects had never used condom.
- A majority of the subjects (85%) reported that there was either no slippage or tearing of condom during last 10 times they used condom during sex.
- About 27% of FSWs in E.Godavari district and 42% of FSWs in Khammam district preferred vaginal microbicides to male condoms, however more than half (71.5% E.Godavari, 42% Khammam), of the subjects preferred to use both male condom and Vaginal microbicides during sex.
- Almost all the FSWs interviewed said that it was their own decision to use vaginal microbicides.
- A majority of the FSWs (76% - 79%) preferred to use vaginal microbicides during peno-vaginal sex rather than any other type of sex, and mostly with non regular customers.
- Almost all the FSWs perceived the importance of using vaginal microbicides and expressed their willingness to participate in the research study on microbicides for prevention of HIV and STI.
- The prevalence of HIV was 15.9% and 10.4% in E.Godavari and Khammam districts respectively, similarly the prevalence of syphilis was 4% and 3.7% respectively.

4 NUTRITION PROFILE OF CHENCHUS - A PRIMITIVE TRIBE OF ANDHRA PRADESH

The tribal population in Indian language known as “Adivasis” stands for original inhabitants, constitutes and they 8.2% of the total of population of India. A general feature of tribal population of the country is their exclusive geographical habitat. There are certain communities among them, who live more or less in total isolation in a lifestyle, which shows only a little change from that of centuries ago. Most of them are small communities with relatively low population growth rate compared to rest of the population. Government of India identified a total of 72 such tribal communities, as primitive tribes on the basis of low population growth rate, primitive agricultural practices, extremely low level of literacy. The Chenchu is one of those primitive tribes inhabiting Nallamala forest area of Andhra Pradesh.

Genesis of the study

Tribal population in Andhra Pradesh constitutes about 6.6% of the total population of the State. There are 12 primitive tribal groups (PTG) in Andhra Pradesh and Chenchu is one of the PTG recognized by Government of India. Chenchus are mainly living in the districts of Mahaboobnagar,

Nalgonda, Prakasam, Guntur, Kurnool, and Ranga Reddy. The formation Rajiv Gandhi Tiger Sanctuary in Srisailam area has led to eviction of tribes from their original home land, as a result the Chenchus are struggling to adapt a new pattern of life as the forest resources are dwindling with time and displacement. An eco-development committee was constituted and had been working with an objective to minimize the tribal depend on forest resources and promoting income generating programmes for their livelihood.

Geographical isolation, primitive agricultural practices, socio-cultural taboos, lack of formal education, poor infrastructural facilities, improper health seeking behaviour, poverty etc., leads to the development of undernutrition and morbidities among tribal population. Earlier reports revealed that Chenchus were under starvation. This leads to undernutrition and higher prevalence of tuberculosis and malaria in the population. The Infant Mortality Rate was 215 per 1000 live births and the maternal mortality rate was 700 per one lakh live births. There are no systematic studies available on 'diet and nutritional status of Chenchus'. Hence, the present study was carried out to assess the 'diet and nutritional status of Chenchu tribal population in ITDA project Srisailam area, spreading over in the districts of Mahaboobnagar, Nalgonda, Prakasam, Guntur, Kurnool and Ranga Reddy.

OBJECTIVES

- To assess 'Diet and Nutritional Status of Chenchu Tribal Community' in the ITDA area of Srisailam.
- To assess household demographic and socio-economic factors
- To assess food and nutrient intake at household level
- To assess nutritional status of community in terms anthropometry and clinical examination for nutritional deficiency signs
- To assess mortality during previous one year in the households and to identify cause of death by verbal autopsy and/or hospital autopsy
- To assess health seeking behaviour among the population
- To assess the infant and young child feeding practices and
- To identify the operational aspects of various developmental programmes

METHODOLOGY

Estimation of Sample size

Assuming the overall prevalence of underweight was 51% among tribal preschool children (NNMB 2010) with 95% confidence interval 10% of relative precision, and with design effect of 1.5 the estimated sample size was 563.

Sampling Procedure

List of Chenchu gudems under ITDA area was obtained from project officer, ITDA, Srisailam. A total of 42 villages were covered for the survey in the ITDA area through systematic random sampling procedure. All the HHs, where index child below five years of age is available in the village was covered for the present survey.

Investigations

The following investigations were carried out in the study

- Household demographic and socio-economic particulars
- A one day 24-hour recall method of diet survey was carried out in a sub sample of HHs to assess

Particulars of Chenchu habitations covered

Sl. No	District	Total No. of Mandals	Total No. of villages	No. of Villages to Covered for study
1	Mahaboob Nagar	10	123	19
2	Prakasam	7	81	9
3	Kurnool	12	40	6
4	Guntur	3	48	8
Pooled		**32**	**292**	**42**

the food and nutrient intake at household level.

- The nutritional status of all the available individuals was assessed through anthropometry such as height and weight and clinical examination for nutritional deficiency signs.
- Information on history of morbidity among the subjects, such as fever, acute respiratory infection, diarrhoea etc., if any, during the preceding 15 days of visit was collected.
- Maternal particulars such as antenatal care, TT immunization, receipt of IFA tablets, particulars of delivery and recording of birth weight were collected on mothers of <6 months children.
- infant and young child feeding practices such as initiation of breast feeding, feeding of colostrum, duration of exclusive breast feeding for under 6 months of children and particulars of complementary feeding in terms of age of initiation, the type of complementary food and frequency of feeding.
- Particulars of coverage of children for all the immunizations such as BCG, DPT, Polio and measles during first year of life was collected for children aged 12-24 months. In addition, the information on coverage of 9-59 months children for supplementation of massive dose vitamin A was also covered.
- Information on health seeking behaviour and utilization of health services was obtained using structured format.
- Village level information on mortality with cause of death was assessed using verbal autopsy.

SALIENT FINDINGS

Coverage

A total of 42 Chenchu gudems from four districts namely Mahaboobnagar, Prakasam, Kurnool and Guntur were covered for the present study. A total of 416 HHs were covered for household socio-economic particulars, and among them a sub sample of 100 households were covered for diet survey, and 307 HHs were covered for information on availability and utilization of health facilities. Two hundred and twenty two mothers of less than three year children were contacted for infant and young child feeding practices.

A total 1396 individuals including all the age groups were covered for anthropometric measurements such as height and weight and for signs nutritional deficiency. In addition, history of morbidity for the previous fortnight was also collected on all these individuals. Village level information on mortality during past one year with cause of death was also collected from all the 42 Chenchu gudems using verbal autopsy.

Socio-economic particulars

About 83% of the HHs covered were nuclear families. The average family size was 4.5. About 40% of fathers and 26% of mothers of index children were literate. The major occupation of the father (93%) and mothers (85%) of the index child was labour. About 4% of fathers and 3% of mothers were doing private /govt. service. Less than 1% each were engaged in agriculture. The

average monthly per capita income (PCI) of HHs was Rs 1333. Contribution of income from different source indicated that 84% per cent of income was from wages, 15% from agriculture/forest produce, while 1% was from livestock.

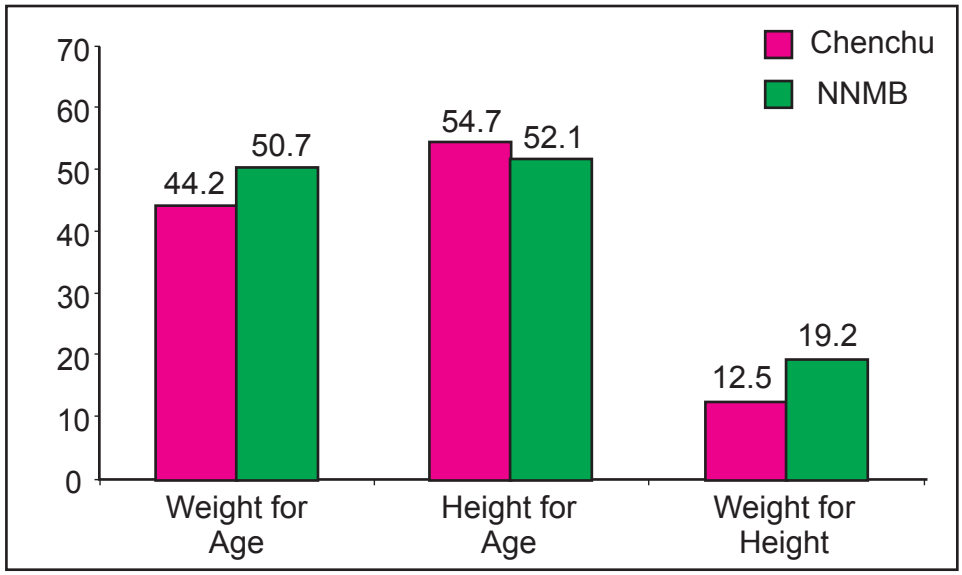
Food and nutrient intakes

Barring cereals, the intake of all foodstuffs were lower when compared to balanced diets. Similarly the intake of all nutrients was also below the recommended levels. The extent deficit in the intakes was relatively higher with respect to micronutrients such as vitamin A, iron, riboflavin, free folic acid.

Nutritional status

The prevalence of underweight, stunting and wasting among preschool children was 44%, 55% and 13% respectively. The prevalence was lower or comparable to their other tribal counterparts of AP (50.7%, 52.1%, 19.2% respectively) (NNMB 2009). It was observed that about 41% of adult men and 42% of adult women had chronic energy deficiency (BMI<18.5). Higher prevalence of conjunctival xerosis (0.7%) and bitot spots (0.9%) indicated that vitamin A deficiency among preschool children is a public health problem.

Fig 1. Prevalence (%) of undernutrition among Preschool Children according to SD Classification (<Median-2SD)



Ante natal care

About 95% of pregnant woman had reportedly undergone antenatal checkups (ANC) during the last pregnancy. About 55% of mothers had undergone at least three ANCs. Seventy one per cent pregnant women registered for ANC before 16 weeks of gestation. In about 79% of mothers, the ANCs were conducted by either Medical officers (govt/pvt) or 16% by ANM or LHV. Ninety seven per cent of the mothers reportedly received TT immunization. About 68% of women received ≥90 tablets; about 42% consumed ≥90 tablets during the pregnancy.

Infant and young child feeding practices

About 62% were institutional deliveries, either at PHC/Govt. Hospital (39%), or private hospitals (23%). A majority of the deliveries were conducted by a Medical officer at PHC (38%), LHV/ANM/TBA (1.9%), and untrained dais/elders (29.6%). As per the records, 16% of the newborn

babies had birth weight of < 2.5 kg termed as low birth weight.

About 23% of the women reportedly gave pre-lacteal feeds to the newborn, which included mostly glucose water, honey or cow/goat milk, etc**.** About 28% of the newborns were given breast feeding within an hour of delivery, while 43% were fed between 1-3 hours. Eighty seven percent of the mothers reportedly fed colostrums to the newborn.

Among the children (<12 months) initiated complementary feeding 7% initiated before 6 months, 20% initiated at 6 months of age, while, 33% initiated after 6 months of age. The commonly used complementary foods included homemade solids (39%), followed by cow/buffalo milk (24%), homemade solids (23%), commercial baby foods (14%) and formula milk (1%).

About 83% of the children were fully immunized, 12% were partially immunized, while about 4% did not receive any immunization. In general, about 62% of 12-35 months children reportedly received at least one dose of vitamin A.

Cause of death using verbal autopsy

A total of 68 deaths were reported during previous one year among the Chenchu community surveyed. Of these, 6 were infant deaths, 7 were school aged and adolescents, 55 were adults. The major cause of deaths among infants was premature delivery and low birth weight.

Among school age and adolescents gastroenteritis and accidents form major cause of death. Among adults cirrhosis of liver, poisoning of alcohol, pulmonary tuberculosis form the major causes of death followed by suicidal deaths, snake bites, accidents, stroke and old age. None of the death attributed to starvation.

CONCLUSIONS

The food basket of Chenchu mainly consists of rice. The consumption of protective foods such as GLV, other vegetables, milk, fats and oils, sugar and jaggery was below the recommended levels causing micronutrient deficiencies such as iron deficiency anaemia among children and women, and vitamin A deficiency in young children.

The prevalence of undernutrition was similar with other general tribes as well as rural counterparts. The better access and availability of food and purchasing power through programmes such as targeted public distribution system, rural employment guarantee programme and other nutrition intervention programmes, to some extent could have averted severe forms of under nutrition in the population. Higher percent of home deliveries and low birth weight, feeding of pre-lacteals to new born, exclusive breast feeding for longer period than desired, delayed complementary feeding and sub-optimal complementary feeding in terms of type of feed, quantity and frequency, frequent morbidity etc, are some of factors associated with undernutrition among children.

Higher rates of illiteracy, cultural taboos, lack of early and irregular treatment of morbidities, seemed to have aggravated the situation. Cirrhosis of liver, illicit liquor consumption, pulmonary tuberculosis forms the major causes of death among adults.

RECOMMENDATIONS

- Currently, 84% of the HHs were benefitted by the targeted PDS (one rupee kilo rice scheme) and the same needs to be extended to all the HHs to improve food security and traditional millets such as ragi and jowar also should be supplied through PDS.
- National Rural Employment Guarantee Programme needs to be strengthened and provide regular employment especially during lean periods in the year.

- Since, forest economy played an important role in their livelihood, small scale industries at household level such as basket making, collection of honey, gum, tamarind, preparation of broom sticks and collection of aromatic roots and tubers such as “sugandhagaddalu”, “palagaddalu” and other forest products, should be encouraged further and necessary arrangements should be made locally through Girijan co-operative Society for purchase the same for the benefit of tribes.
- Encourage home gardens for green leafy vegetables and other vegetables to be grown in their backyard to improve their consumption in order to control micronutrient deficiencies.
- Horticulture also needs to be encouraged, which will improve their economy.
- Cattle rearing should be encouraged among settled Chenchus.
- Needs to improve the health care both in availability and utilization of the same by regular home visits by health personnel.
- Awareness campaign should be organised by strengthening IEC activities on various national programmes explaining the benefits of each scheme.
- Referral fund should be provided to the anganwadi centres in order to refer any emergency cases/ sick patients, if any, to the nearest health centre.

5 ASSESSMENT OF NUTRITIONAL STATUS OF BELOW FIVE YEAR RURAL CHILDREN AND PERFORMANCE OF ICDS FUNCTIONARIES IN THE DISTRICTS OF GUJARAT STATE

Despite rapid growth in agricultural and industrial sector, and consequent to economic development, undernutrition continues to be a major public health problem in the developing world, including India. Though, poverty is a major underlying cause, scores of other factors such as socio-demographic, socio-cultural and lifestyle practices contribute significantly to the problem of malnutrition.

According to NNMB survey (2012) carried out in the rural areas of the Gujarat State, about 53% children of <5 year were underweight, 57% were stunted and 25% were wasted (WHO standards). The corresponding figures reported by NFHS-31 for the Gujarat State were 44.6%, 51.7% and 18.7% for underweight, stunting and wasting respectively.

Several nutrition intervention programmes such as supplementary feeding through ICDS for prevention and control of undernutrition among vulnerable groups, distribution of iron and folic acid tablets, massive dose vitamin A supplementation, mid-day meal programme etc are being implemented in India, through respective State Governments, during the past few decades for the prevention and control of both macro and micronutrient malnutrition in the population. Also, several poverty alleviation programmes such as Public Distribution System (PDS) including TPDS, National rural employment guarantee programme (NREGP) etc, especially to those below poverty line throughout the year, all over the country, to ensure household food security are being implemented by central and State governments, for the overall socio-economic development of the communities. Still undernutrition continues to be major nutritional problem in India.

The Government of Gujarat is contemplating to develop state and district Nutrition Policy and develop plan of action for implementation in priority districts, in order to improve the nutritional status of the communities. Therefore, at the request of the Department of Women and Child Development, Government of Gujarat, the National Institute of Nutrition carried out the present survey in all the following 26 districts of the State, to assess the nutritional status of under 5 year children and IYCF practices.

OBJECTIVES

To assess the health and nutritional status of <5 year children and infant and infant and young child feeding (IYCF) practices among <3 year children in the rural areas at the district level.

The specific objectives of the study were

- To assess the nutritional status of<5 year children in terms of anthropometry such as height and weight, and prevalence of clinical signs of nutritional deficiency
- To assess the prevalence of morbidity among <5 year children during previous fortnight
- To assess the IYCF practices among mothers of <3 year children
- To estimate the iodine levels in the household (HHs) salt samples used for cooking by spot testing kit, and
- To assess knowledge, practice and performance of various functionaries of ICDS.

METHODOLOGY

Sampling design

It was a cross-sectional, community based study carried out by adopting systematic sampling procedure.

Sample size

Assuming an overall prevalence of underweight (weight for age <Median-2SD of WHO standards) as 46% among <5 year children, with 5% absolute precision and 95% confidence interval (CI), a sample size of 375 (or 400) children per district is arrived at.

INVESTIGATIONS

- Household demographic and socio-economic particulars
- Anthropometric measurements such as height and weight
- Clinical examination for identification of nutrition deficiency signs
- History of morbidity during the preceding 15 days
- Maternal particulars such as parity, antenatal care, TT immunization, receipt of IFA tablets and supplementary foods during pregnancy
- Knowledge and practices of mothers on infant and young child feeding practices
- Coverage of children under various health and nutrition intervention programmes
- Spot testing of household cooking salt for iodine

Salient findings of the study

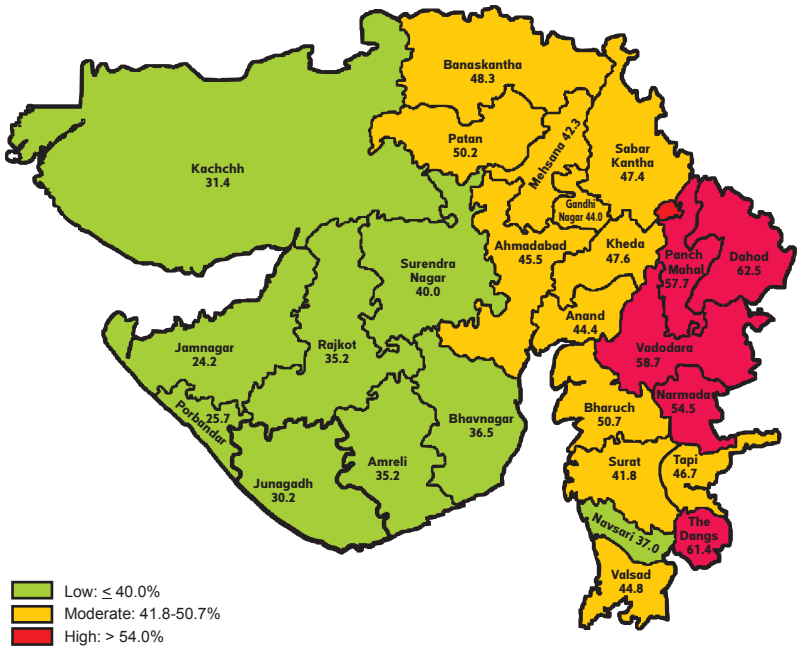
A total of 12,969 children (Boys: 52.3%) of <5 year age old were covered from the selected 10424 households in 520 Anganwadi centers for the various investigations such as anthropometry, clinical examination of nutritional deficiency signs, history of morbidity and food preferences, taboos during health and disease etc. The study revealed that about 31% infants received breastfeeding within

one hour of birth, about 75% children of 6 -11 months, received complementary feeding (CF) in addition to breast milk, while only 20% received CF at 6 months of age. About 90% of the children of 6-59 months age group were participating in the ICDS supplementary feeding programme, with 65% being regular. About 86.5% of the children were fully immunized, while 10% were partially immunized and 1% did not receive any vaccination. About 87% of 12-59 months children reportedly received at least one dose of vitamin A during the previous year.

Mapping of undernutrition

Identification of geographical areas with similarities in the extent of problem of undernutrition and factors contributing to the same is essential to develop and implement region specific intervention strategies. For this purpose, cluster analysis was carried out to identify three geographical areas/ groups of districts in the state, with extent of underweight (high, medium and low) among children of <5 years as criterion. After Identification of geographical areas/districts various socio-economic and demographic characteristics, infant and young child feeding practices, coverage under health and nutrition interventions etc., were studied for each geographical region/ group of districts (clusters).

Mapping of Districts according to the magnitude of Underweight* (weight for age <Median-2SD)



Nutritional status of <5yr children * WHO standards used Gujarat districts pooled

The cluster analysis resulted in grouping of 5 districts in cluster 1, 12 districts in cluster 2 and 9 districts in cluster 3 (Fig 3). The overall prevalence of underweight was 59% in cluster 1, 46% in cluster 2 and 33% in cluster-3. Similarly, the overall prevalence of stunting was significantly ($p<0.01$) higher in cluster 1 (52%), followed by cluster-2 (42.8%), with lowest in cluster-3 (36.1%). The overall prevalence of wasting was 30.6% in cluster 1, 19.8% in cluster 2 and 13.1% in cluster-3 and inter-cluster differentials were statistically significant ($p<0.01$) Fig.2 & 3.

It was observed that the proportion of children belonging to SC/ST population, joint families, illiterate parents, households having agricultural land and major occupation of parents being labour

Fig. 1 Time of initiated breast feeding after delivery by the mothers

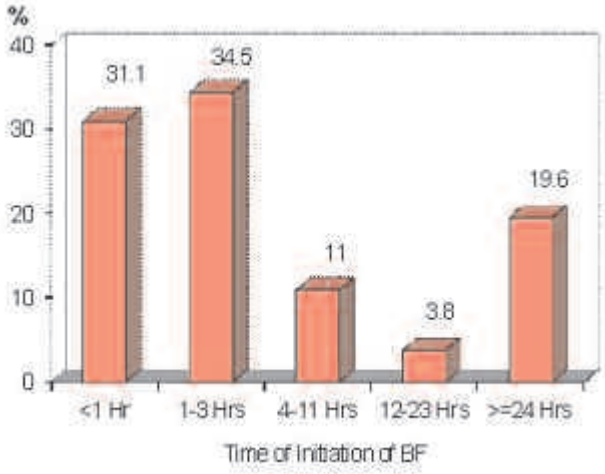
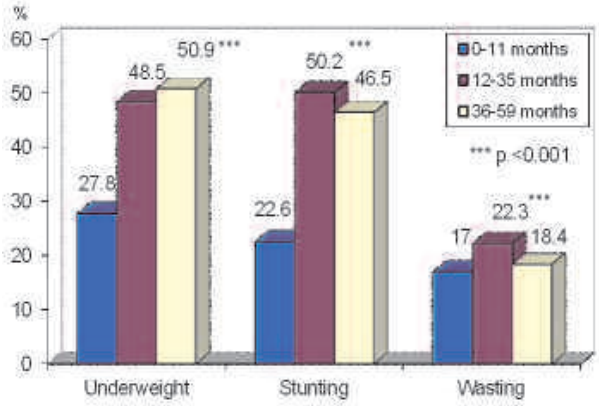


Fig. 2 Prevalence (%) of Undernutrition among <5 yrs Children according to SD Classification (<Median – 2SD) **by Age group**



was significantly ($p<0.01$) higher in cluster 1. The proportion of children belonging to households having physical facilities such as possessing pucca house, separate kitchen, access to safe drinking water, presence of electricity and presence and usage of sanitary latrine was significantly ($p<0.01$) lower in cluster 1 compared to other clusters. The indicators of health seeking behaviours during pregnancy and delivery such as having ANC check up and having $\geq$3 ANC visits, extent of early registration (<16 weeks of gestation) for ANC, receipt of TT immunization, consumption of IFA tablets ($\geq$90) and institutional deliveries during last pregnancy were significantly ($p<0.01$) low, whereas low birth weight was higher in cluster 1. Optimal infant and young child feeding (IYCF) practices such as exclusive breast feeding up to 6 months and washing hands before feeding the child were significantly ($p<0.01$) low in cluster 1. The proportion of children covered for vitamin A supplementation (VAS) and fully Immunization during and participation in ICDS programme was significantly ($p<0.01$) low in cluster 1, while the use of ORS during diarrhoea episodes was significantly higher ($p<0.01$) in cluster 1 compared to others.

Thus, the study indicates that there is a need to improve the above aspects, with special emphasis on the 5 districts in cluster 1.

II BIOSTATISTICS AND BIOINFORMATICS

1 IDENTIFICATION OF BEHAVIORAL PATTERNS AND STUDY OF THEIR RELATIONSHIP WITH HIV STATUS AMONG FEMALE SEX WORKERS - BY USING FACTOR ANALYSIS

India, the second-most populous country in the World, has been experiencing a highly varied HIV epidemic, in the past two decades. HIV in India is concentrated mainly among 'high-risk' populations such as Injecting Drug Users (IDUs), Men having Sex with Men (MSM) and Female Sex Workers (FSWs). Andhra Pradesh state remains to be critical in the spread and control of the epidemic. The state has the second highest estimated adult HIV prevalence of 0.90% after Manipur (at 1.4%).

HIV prevalence among Female Sex Workers (FSW) in Andhra Pradesh increased from 7.3% in 2006 to 9.7% in 2007. Targeted Interventions by the Andhra Pradesh AIDS Control Society (APSACS) and *Avahan* by Bill and Melinda Gates Foundation (BMGF) are the two major HIV/AIDS prevention programs currently in operation in the State. The State government (in partnership with NACO) has been implementing prevention, care and treatment programs since 1999, which have resulted in significant gains.

In this backdrop the present analysis proposes to utilize the 'Integrated Behavioral and Biological Assessment' (IBBA) data to analyze and identify the demographic, behavioural and biological patterns among FSWs that contribute or relate to the HIV prevalence in the State of Andhra Pradesh.

OBJECTIVE

To identify behavioral patterns of FSWs through factor analysis and study their relationship with HIV status.

Source of data

The data collected in the study entitled “Mapping, Size Estimation and Integrated Behavioral and Biological Assessment (IBBA) in high HIV prevalence settings in India –Round 2” carried out by the division of community studies during 2010 will be utilized.

METHODOLOGY

Coverage

A total of 3083 FSWs were covered from 8 districts in the State, namely Hyderabad, Karimnagar and Warangal, Visakhapatnam, East Godavari, Guntur, Prakasam and Chittoor.

Variables considered

The potential risk variables considered in the present analysis included demographic variables such as 1) age 2) literacy, 3) Marital status 4) Age of initiation of sex work and Duration in sex work, the behavioral variables such as 1) Having regular clients, 2) Having regular unpaid partners, 3) Having other regular unpaid partners, 4) Having occasional clients, 5) Wanted to use condom but could not use due to several reasons, 6) Dependency on sex work, 7) Had sex during travel, 8) Client volume per week, 9) Carrying condom at the time of interview, 10) Exposed to any interventions, 11) Correct knowledge of HIV/AIDS, 12) Obtained condom from Peer Educator and the biological parameters such as 1) History of having any STI during past 12 months, and Suffering from any STI (Syphilis or NG or CT) at the time of interview.

Statistical analysis

The percentage distributions of different demographic, behavioral and biological variables will be calculated. Behavioral patterns were obtained by exploratory factor analysis for 20 potential risk variables considering simultaneously. The principal component analysis (PCA) technique, followed by factor analysis will be used to transform a large set of correlated variables into smaller sets of non-correlated variables, called principal components or factors. The factor analysis of the data objectively points out characteristics those are clustered. The aim of this technique is to identify the underlying structure in data matrix, by summarizing and consigning data to arrive at a systematic measurement of the behavior. To summarize data, factor analysis requires dimension that, when interpreted and understood, describes it in terms of a much smaller number of significant variables.

For the applicability of factor analysis, the uniformity of sample will be tested by examining the distribution of variables in a loading plot, contrasting the value observed against those expected in a normal distribution which was verified by Kaiser-Meyer-Olkin (KMO) measurement of adequacy. A KMO value of more than 0.50 was considered acceptable. The presence of correlations between demographic, behavioral and biological variables was tested using the Bartlett test of sphericity (homogeneity of variance). Principle Component analysis will be used for extraction of factors and orthogonal rotation (Varimax option) to derive non-correlated factors (4), to minimize the number of indicators that have high loading on one factor (5). The first factor extracted is the one that accounts for the maximum possible variance in the dataset. The second component, independent of the first, will be the one that explains the largest possible share of the remaining variance and so on, without the components being correlated with each other (6). While deriving behavioral patterns for FSWs dataset, the variable, viz., current HIV status will not be included in the analysis as it is intended to study the relationship between behavioral patterns vis-à-vis HIV status.

Cluster analysis will be performed to identify relatively homogeneous groups of cases based on the factor scores derived through factor analysis, using an algorithm that can handle large number of cases. However, number of clusters would depend on the number of cases fall in different clusters. The prevalence of HIV was considered to name and label the cluster. Correctness of labeling of cluster will be verified by testing the significant difference between the clusters on the back ground characteristics of the cases.

RESULTS

The distribution of FSWs according to demographic and behavioral characteristics is presented in Table 1. Majority (42%) of FSWs solicit their clients in public places. About 19% FSWs were in the age group of <25 years, about 47% were in the age group of 25-34, while 35% were in the age group of 35+ years. Majority (50%) of FSWs started commercial sex work before 25 years about 50% reported a duration of >5years of sex work. While half of the FSWs reported that their volume of clients was >10 per week.

About 42% of FSWs were literates and 56% were married and staying with their family. Majority of FSWs had regular unpaid partners (72.8%) and regular clients (83.3%). Majority of the FSWs (81%) reported that they were using condom consistently with occasional clients, about 17.2% reported that they wanted to use condom but could not use due to various reasons and very few (7.7%) reported had sex during last travel. About 47% of FSWs were carrying condom at the time of survey, while 61% reported that they obtained the condom from peer educator. About 33% of FSWs reported the history of having any STI (NG, CT, and Syphilis), while 10.1% were currently suffering from STIs. Only 1% of the FSWs had correct knowledge on HIV. While majority of FSWs (73.5%) reportedly undergone HIV testing, 11.4% of them were HIV positive.

Table 1. Frequency (%) distribution of FSWs according to their demographic, Behavioral and biological profile (n=3083)

Variable	Category	%
Age (years)	<25	18.7
	25-29	26.1
	30-34	20.7
	35+	34.6
Age at start of sex work (years)	<20	18.2
	20-24	32.0
	25-29	26.4
	30+	23.4
Duration of Sex work (years)	0-1	14.6
	2-4	35.9
	5-9	30.4
	10+	19.1
Client Volume per week	<5	10.4
	5-9	38.1
	10+	51.5
Usual place of solicitation	Home	38.3
	Brothel/Lodge/Daba	20.3
	Public Places	41.5
Literacy Status	Literate	42.3
Depend on sex work	Yes	49.3
Have other regular un paid partners	Yes	72.8
Had sex during travel	Yes	7.7
Having regular clients	Yes	83.3
Having unpaid regular partners	Yes	72.8
Consistent condom use with Occasional clients	Yes	80.9
Wanted to use condom but could not use		17.2
Carrying condom at the time of interview		46.6
Obtained condom from Peer Educator		60.8
Exposed to intervention		75.8
Ever taken HIV test		73.5
History of having STI during past 12 months		32.7
HIV positive		11.4

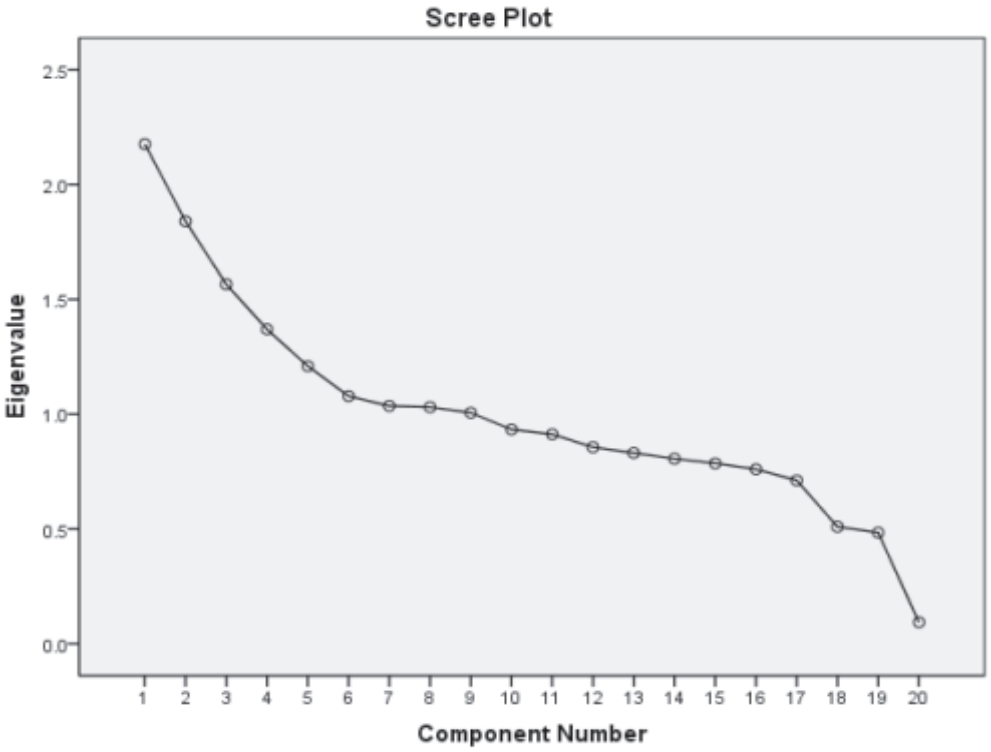
Analysis reveals that nine components for high risk population were extracted by factor analysis using Principal Component Analysis with varimax rotation for demographic, socioeconomic and behavioral variables (Table 2). These nine components (factors) in the initial solution have an Eigen value over 1, and they account for about 62% of the observed variation in the behavioral pattern among female sex workers. The parallel line to horizontal at Eigen value equaling to 1 in scree plot showed that nine factors will be extracted for this population (Fig.1).

The first factor, which accounted for 10.9% of the total variance among the female sex workers was labeled as unpaid regular partner. High factor loading observed for those FSWs who are either married or having a regular unpaid male partner characterized these factors. The second factor explained 9.2% of the total variance and was labeled as exposure to intervention (Exposed to any type of intervention and obtained condom from peer educated employed by the NGOs working in that area).

The third factor which accounted for 7.8% of the total variance was characterized by the age of the FSW and duration in sex work was labeled as age and duration of sex work. The fourth factor explained 6.8% of the total variance was done labeled as correct knowledge of HIV/AIDS and getting HIV testing done voluntarily. The fifth factor accounted for 6.0% of the total variance and characterized having any STIs for the past 12 months and wanted to use condom but could not use and it was labeled as suffering from STIs. The sixth factor explained 5.4% of the total variance and was labeled as dependency (majorly depend up on sex work and the place of solicitation).

The seventh factor accounted for 5.2% of the total variance and was characterized by single variable namely having regular clients who pay every time which was labeled as regular clients. The eighth factor also explained 5.2% of the total variance and was

Fig. 1. Socio-demographic, Behavioural and Biological characteristics of FSWs



Scree plot showing Eigen values for each of the Socio-demographic, Behavioural and Biological components, extracted by factor analysis data collected through IBBA Round-2.

Components

1. Having unpaid regular parteners	2. Exposure to any intervention	3. Age & duration of sex work
4. Correct knowledge	5. Suffering from STI's	6. Depend upon sex work
7. Regular clients	8. Volume of occasional clients	9. Positive for STI's

labeled as having occasional clients (having occasional clients and the volume of these occasional clients per week). The ninth and the last factor explained 5.0% of the total variance and was labeled as positive for STIs (at the time of survey positive).

In the present analysis, only two clusters were formed with sizeable numbers in each of the cluster for further analysis. Cluster 1 was formed with 2668 subjects and cluster 2 with 415 subjects. The distributions of the FSWs according to their back ground characteristics in these two clusters were tabulated and look for significant difference in the proportions are presented in Table-3. The data revealed significant ($p<0.01$) differences between clusters (formed on the basis of behavioral patterns) with regard to place of solicitation, age, age at start of sex work, duration of sex work, client volume, literacy status, having sex during travel, having regular clients, unpaid regular partners, consistent condom use with occasional clients, exposure to intervention, carrying condom at the time of interview, obtained condom from peer educator and wanted to use condom but could not use due to various reasons.

Also significant differences exist between clusters with respect to proportion of those with history of having any STI (NG, CT, Syphilis) during last one year, presently suffering from any STIs, and with HIV positivity. These findings indicate that the FSWs of cluster-2 had different socio-demographic, behavioral characteristics which are likely factors for STI and HIV positivity. The

Table 2. Rotated Component Matrix for Socio-demographic, Behavioral and Biological parameters

Rotated Component Matrix[a]

	Component								
	1	2	3	4	5	6	7	8	9
Had sex during travel	-.087	-.263	.202	.329	.199	.019	.425	.021	.110
Obtained condom from PE	.005	.774	.048	.074	-.135	.005	.093	-.001	.021
Wanted to use condom but could not	-.001	-.211	.169	-.078	.573	.273	-.079	-.042	-.019
Have occasional clients	.034	.153	.011	.266	.020	.038	-.462	.546	.021
Have Regular clients	.047	.186	.036	-.005	-.060	-.080	.680	.086	-.108
Have Regular unpaid partners	.971	.035	-.013	.020	.031	.017	.026	-.001	-.018
Have other regular unpaid partners	.109	-.212	-.188	.187	.399	-.007	.084	.272	-.153
Ever taken HIV test	.019	.243	.066	.642	.077	-.006	-.011	-.068	-.004
Literate	.021	-.196	-.295	.311	-.056	-.492	.173	-.035	-.155
Dependent on sex work	.088	.140	-.043	.001	.255	.611	.169	-.016	-.238
Solicitation Place	-.021	-.227	-.065	.261	-.298	.642	-.070	.028	.135
Carrying condom	.010	.308	-.059	.013	-.044	.216	.423	.079	.175
Had any STI in past 12 months	.003	.107	-.126	.066	.723	-.121	-.022	-.036	.121
Any STI (Syph or NG or CT)	-.021	-.006	-.019	-.007	.056	-.009	.002	.002	.916
Exposed to any intervention	.078	.719	.096	.272	.084	.019	.097	.095	-.057
Marital Status	.972	.039	.000	.037	.013	.024	-.007	.001	-.015
Correct knowledge of HIV/AIDS	.035	.073	-.073	.720	-.025	.040	.001	.074	-.007
Age	-.012	.101	.808	-.033	.001	.184	-.079	-.056	-.097
Clients per week	-.027	.034	.030	-.090	-.025	.004	.191	.831	.010
Duration in Sex work	.006	.008	.776	.037	-.077	-.166	.157	.073	.063
Solicitation Place	-.021	-.227	-.065	.261	-.298	.642	-.070	.028	.135

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization
a. Rotation converged in 16 iterations

Table 3. Distribution (%) of FSWs by their background characteristics by clusters 1 & 2

Variable	Category	Cluster -1 (n=2668)	Cluster-2 (n=415)
Age (years) **	<25	19.1	15.9
	25-29	26.7	22.2
	30-34	21.0	18.3
	35+	33.2	43.6
Duration of Sex work (years) **	0-1	15.9	5.8
	2-4	37.1	28.0
	5-9	30.2	31.8
	10+	16.8	34.5
Age at start of sex work (years) **	<20	17.2	24.3
	20-24	31.7	34.0
	25-29	26.8	23.5
	30+	24.2	18.3
Client Volume per week **	<5	9.2	18.4
	5-9	38.9	32.9
	10+	51.9	48.8
Typology **	Home Based	19.7	19.5
	Brothel/Lodge Dhabha	9.3	11.6
	Public Place	71.0	68.9
Literacy Status	Yes	41.5	48.0**
Depend up on sex work	Yes	49.6	48.0
Having other regular un paid partners	Yes	23.0	22.7
Had sex during travel	Yes	1	57**
Having regular clients	Yes	82	94**
Having unpaid regular partners		74	63**
Consistent condom use with Occasional clients	Yes	82.6	69.9**
Wanted to use condom but could not use during last sex	Yes	16	28**
Carrying condom at the time of interview	Yes	45.5	53.5**
Obtained condom from Peer Educator	Yes	62.3	51.3**

[2] ** Sig p<0.01

Table 4. Classification of subjects with HIV and without HIV using observed HIV and predicted HIV using scores derived from factor analysis for demographic & behavioral characteristics

HIV			Predicted HIV group according to factor scores derived through factor analysis		Total
			-ve	+ve	
According to HIV test	n	-ve	1910	823	2733
		+ve	170	180	350
	%	-ve	69.9	30.1	100
		+ve	48.6	51.4	100

prevalence of HIV positivity was 11.4% among this high risk FSW population. The distributions of FSWs predicted to have HIV positive based on the derived HIV status by discriminant function analysis using the factor scores obtained from factor analysis for various demographic and behavioral characteristics as against those based on the actual tested HIV positivity are presented in Table 4. Based on the discriminant function analysis, an overall, 68% of FSWs with and without HIV positive were predicted correctly by factor scores. In other words, the sensitivity of discriminant function was 51.4%, and the specificity was 70%.

CONCLUSIONS

The data allowed identification of patterns defined by factor analysis based on the data collected from FSWs. There exists a strong relationship between specific behavior patterns and HIV status among the high risk populations. These results will be useful for identifying people in the community with risk behaviors which in turn will help the planners in formulating interventions accordingly.

Expected outcome

The multi-variate statistical analysis will identify the risk behaviors among FSWs and also relationship between risk behaviors and HIV status. These results will be useful for identifying people in the community with high risk behaviors and also will help the planners in formulating interventions accordingly.

2 DEVELOPMENT OF COMPOSITE INDEX AND RANKING THE DISTRICTS IN THE STATE OF MADHYA PRADESH

United Nations annually ranks all the member countries on the basis of health, education and income which are the three aspects of human development. The human development index decides the relative rank of a country's achievement in a concise manner. This helps to identify the countries of immediate concerns as well as prioritize the relevant policy areas. National level figure of the index has its own limitations in the policy formulation, especially, for a large country like India where socio-cultural, demographic and socio-economic status is diversified. To minimize such limitations, it needs to adopt a measure that can capture the said disparities in the aspects of human well being where appropriate policy actions are urgent at the level of the smallest possible administrative unit. The 73rd and 74th amendments of the constitution and Reproductive and Child Health approach have emphasized the need of decentralization, and therefore districts become the focus for planning and programme implementation.

In backdrop of this, the assessment of the current status of developmental districts of India is essential and districts vary considerably in the achievement of socio-demographic, health, social status and development of infrastructure. Therefore, the ranking and mapping of the districts on the basis of the selected developmental indicators will help to identify the backward districts with their relative performance in the State, so that appropriate strategies can be developed to address the issues of low performance.

Sources of data

To carry out the proposed analysis, database of Madhya Pradesh district Nutrition survey (2010) entitled "To assess the health and nutritional status of <5 year children and infant and young child feeding practices among <3 year children in the rural areas at the district level in the State of Madhya Pradesh" and District level Health Survey (DLHS-2) were utilized.

OBJECTIVE

To rank and map the districts on the basis of selected developmental indicators vis-a-vis undernutrion in the State of Madhya Pradesh.

Variables will be included in the proposed analysis (in %):

- Female Literacy Rate
- Children (12-24months) fully immunized
- Household access to safe drinking water
- Household having Toilet Facility
- HHs with Electricity
- Received 2 doses of TT injections during pregnancy
- Pregnant women had ≥3 or more ANCs
- Children with birth order ≥3
- Women with maternal age < 20 years
- Children with Partially immunized
- Population <5 years (DLHS-2)
- Under 5 mortality rate (DLHS-2)
- Prevalence of Underweight among < 5years.

METHODOLOGY

Coverage

A total of 22,907 <5year children (Boys: 12,387; Girls: 10,520) covered from 19,756 households in 1000 villages covered from all the 50 districts of Madhya Pradesh.

Statistical analysis

Iyengar and Sudarshan (1982) attempted to classify regions using multivariate data to measure development. The mechanism has been widely used to provide a composite index for spatial differentials in the level of development. Present analysis will also follow a similar mechanism to calculate the index value for a given development indicator and the composite index value for a district. In brief, it can be defined as follows;

Let Xi represent the value of the i-th development and if Xi is positively associated with development of district, then

$$\text{Index Value (Xi)} = \frac{(Xi) - \text{Min (Xi)}}{\text{Max (Xi)} - \text{Min (Xi)}} \quad \text{----------I}$$

For e.g. The higher the female literacy rate, better is the district.

If X_i is negatively associated with development of district, then

$$\text{Index Value } (X_i) = \frac{\text{Max } (X_i) - (X_i)}{\text{Max } (X_i) - \text{Min } (X_i)} \quad \text{---------- II}$$

For e.g., If non immunized & Partially immunized children are low, better is the district.

The composite index is the simple average of all the indices. One may argue that, among the selected indicators one is more important than the other and therefore, for the composite index, there is a need to give appropriate weights to each of the Indicator. We have opted for a simple average to construct the composite index.

$$\text{Cumulative Index (CI)} = (X_1+X_2+X_3+\ldots\ldots\ldots+X_{13})/13 \quad \text{----------III}$$

After the calculations, the values will be sorted out in descending order accordingly. Higher the value, better the rank, second highest will be second rank, similarly, the last value will get the last rank. The Percentiles will be calculated and used to classify the districts. The index values up to 25th Percentile has been considered in the category of least developed districts. The districts having the composite index value between 25th percentile and 75th percentile are treated as developing districts. Finally, districts with composite index value above 75th percentile are grouped under more developed districts. The ranking of the districts obtained by using data generated by NIN will be compared with that of ranks given by IIPS in 2006 for Madhya Pradesh State.

RESULTS

The descriptive statistics (Minimum, maximum and average percentage) of various parameters included in the analysis are presented in table-1. About a third of children interviewed the birth order was three and more. The maternal age (<18 years) at the time of marriage varies from 0% to 4.1% in a district. A maximum of 65% of the children in a district were partially immunized with an average of 12.12%. The average female literacy is about 48% with a minimum of 13% and maximum of 79%. Majority of HHs (81.04%) were using safe drinking water (bore well and tap). While, the usage of toilet facility was poor (12.12%). About three fourth of the HHs surveyed had the electricity facility. Health parameters such as ANC visits, TT injections and complete immunizations are satisfactory. The prevalence of underweight is comparable with the data generated during the corresponding period for Madhya Pradesh by NNMB.

Table 1. Descriptive statistics of Indicators

S No	Indicator	Min	Max	Mean
1	Birth order three and above	21.0	51.0	35.5
2	Maternal age at the time of marriage (<18 years)	0.0	4.1	0.98
3	Partial Immunization	0.0	65.1	12.68
4	female literacy rate	13.0	78.8	48.43
5	safe drinking water	41.4	99.2	81.04
6	toilet facility	0.8	41.2	12.12
7	Electricity	18.0	97.7	74.69
8	women received >=3 ANC visits	7.9	100.0	36.91
9	women received 2 TT injections	55.3	100.0	86.33
10	complete immunization	32.1	100.0	84.5
11	% of Population 0 -5 years	11.7	20.3	14.8
12	Under 5 Mortality / 1000	51	140	90.8
13	Prev. of Underweight	34.7	67.1	51.8

Calculating Index value of Bhopal district for female literacy indicator Using - Formula I

- Female literacy in Bhopal is : 59.6
- The minimum in MP is: 13.0 (Alirajpur)
- The Maximum in MP is: 78.8 (Balaghat)
- The index value for Bhopal is:

$$\text{Index Value } (X_{flit}) = \frac{(X_{flit}) - \text{Min } (X_{flit})}{\text{Max } (X_{flit}) - \text{Min } (X_{flit})}$$

$$= (59.6 - 13.0) / (78.8 - 13.0)$$

$$= 0.70821$$

Calculating Index value of Bhopal district for maternal age < 20 yrs - using Formula II

- Maternal age (mage) in Bhopal is : 0.5
- The minimum in MP is: 0.0 (Anuppur)

The Maximum in MP is: 4.1 (Indore)

$$\text{Index Value } (X_{mage}) = \frac{\text{Max } (X_{mage}) - (X_{mage})}{\text{Max } (X_{mage}) - \text{Min } (X_{mage})}$$

$$= (4.1 - 0.5) / (4.1 - 0.0) = 0.87805$$

Calculating composite index using formula III

The Composite Index for Bhopal is

$$CI_{Bhopal} = (X_1 + X_2 + X_3 + \ldots\ldots\ldots + X_{13})/13 = 0.7442$$

Similarly, the composite indexes were calculated for all the indicators for all the districts and ranking was given accordingly.

The Composite Index values were divided on the basis of percentiles and presented below. Composite index value and the relative rank of the district was presented in table 2a,2b &2c. Indore district has the highest relative ranking of 1 as compared to other districts. While, singrauli district has the lowest in rankings.

Composite Index
0.3209 to 0.5070 → Under Developed Districts (12)

25th Percentile= 0.5162
0.5193 To 0.6878 → Developing Districts (26)

75th Percentile = 0.6904
0.6980 To 0.7880 → More Developed Districts (12)

Table 2a. Composite index value and Rank of Districts falling in the under developed category

S No	District	CI	Rank
1	Singrauli	0.321	50
2	Umaria	0.3716	49
3	Barwani	0.3927	48
4	Sathna	0.4014	47
5	Alirajpur	0.4107	46
6	Shadol	0.4524	45
7	Tikamgarh	0.4666	44
8	Panna	0.4673	43
9	Sidhi	0.4741	42
10	Jhabua	0.4868	41
11	Rewa	0.4892	40
12	Ashokngar	0.507	39

Table 2b. Developing Category of districts

S No	District	CI	Rank
13	Damoh	0.5193	38
14	Shivpuri	0.5209	37
15	Guna	0.5239	36
16	Sheopur	0.5396	35
17	Rajgarh	0.5454	34
18	Mandla	0.5503	33
19	Chhatarpur	0.5509	32
20	Datia	0.5564	31
21	Dindori	0.5669	30
22	Dhar	0.5693	29
23	Khargone	0.5889	28
24	Katni	0.6085	27
25	Sagar	0.6088	26

Table 2b. Developing Category of districts

S No	District	CI	Rank
26	Vidisha	0.6091	25
27	Morena	0.6099	24
28	Gwalior	0.6163	23
29	Khandwa	0.6179	22
30	Shajapur	0.627	21
31	Bhind	0.6359	20
32	Burhanpur	0.6498	19
33	Sehore	0.6558	18
34	Ratlam	0.6575	17
35	Mandsaur	0.658	16
36	Anuppur	0.6623	15
37	Chindwara	0.6795	14
38	Dewas	0.6878	13

Table 2c. Districts in the more developed category

S No	District	CI	Rank
39	Seoni	0.698	12
40	Raisen	0.6985	11
41	Ujjain	0.7104	10
42	Neemuch	0.712	9
43	Harda	0.7263	8
44	Bhopal	0.7442	7
45	Hoshangabad	0.7468	6
46	Betul	0.751	5
47	Jabalpur	0.7785	4
48	Narasimhapur	0.7817	3
49	Balaghat	0.7841	2
50	Indore	0.7881	1

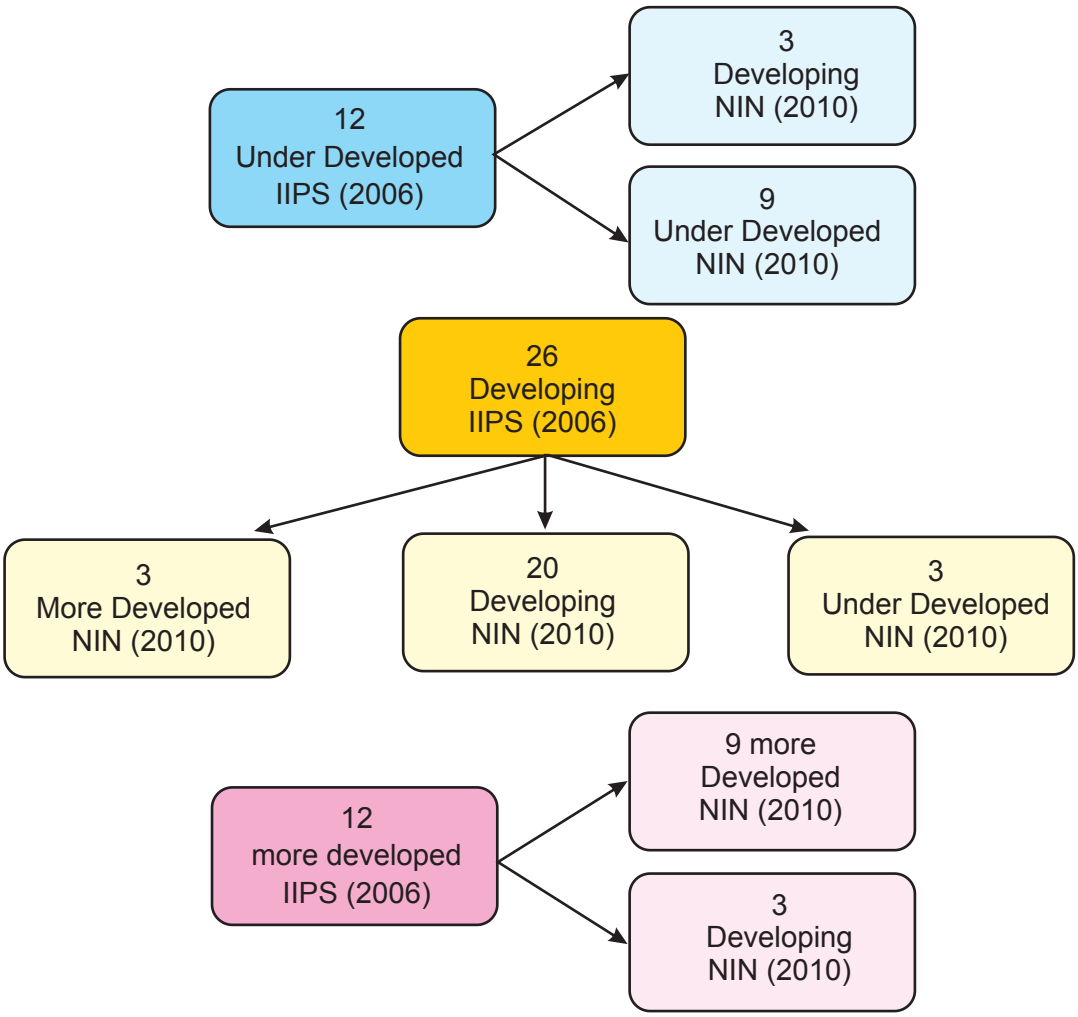
Table 3. Difference in Nutritional status & background characteristics of HHs in three different categories of development

	Under Developed	Developing	Developed
Underweight	57.8 *	51.8	45.8
Stunting	54.7 *	49.8	40.8
Wasting	28.2 *	25.9	23.4
% of SC/ST Population	58.1 *	43.9	43.3
Occupation of mother as labourer	35.5 *	26.4	26.3
Regular participation in ICDS	33.8 *	50.1	58.6
Possess & use of Sanitary latrine	5.5 *	10.3	22.3
HHs Using >15 ppm Iodized salt	34.2 *	41.2	73.2

The prevalence of overall underweight, stunting and wasting was significantly higher in relatively under developed districts as compared to developing and developed districts (table -3). Similarly, significantly higher proportion of SC/ST population, majority of mothers interviewed were laborers', low participation in supplementary nutrition program (ICDS), HHs who possessing & usage of sanitary latrine and lower percentage of HHs usage of iodized salt.

To validate the rankings based on the present survey was compared with that of the ranks given by the Indian Institute of Population studies. Nearly 80% of the districts, the ranks given by the two surveys were similar viz; the 12 districts ranked as under developed by IIPS in 2006, NIN survey ranked 9 districts are under the same category, while 3 districts moves towards the developing category. However among the category of developing districts in 2006, about 12% of districts (3 out of 26) moved in to the category of developed and the similar number of districts moved to relatively under developed districts.

Comparison with IIPS Ranks



CONCLUSION

This cumulative index helps to categorize the districts in different levels of development. It will help the policy makers to divert the limited resources to the needful areas. Need to intensify interventions in the under developed districts, and keep strengthening the existing programmes in the other districts.

3 LATENT GROWTH MODELS FOR DIFFERENT ANTHROPOMETRIC MEASUREMENTS OF 3-36 MONTHS CHILDREN

Linear growth retardation is thought to begin as early as 3-6 months of life and is present even at birth. Stunting is common feature in developing countries by the age of 2 years. Generally, individual growth curve models allow researchers to measure change over time in a phenomenon of interest (e.g., response to treatment) at both the aggregate (i.e., population) and individual (i.e., study participant) levels.

OBJECTIVES

- To study the Shape of the growth curve for each anthropometric measure
- To study the intercept for each anthropometric measure
- To study the slope for each measure.

Data available

A double-blind randomized, controlled clinical trial of Zinc supplementation to full-term infants in Hyderabad urban slums. Cohort followed from 3 to 36 months (n=427). Anthropometric measurements like weight, height, head, chest and mid upper arm circumference and skin fold thicknesses at triceps, biceps, sub scapular, maternal height and weight were collected every three months up to 24months and another measurement was done at 36months.Data regarding feeding practices and any other supplementation was recorded for all the children. Immunization status of all children was recorded at monthly visits. Morbidity data collection was done by 15 day recall method every month.

Diarrheal and respiratory morbidity was collected by 15 day recall as to the number of episodes and the total duration of morbidity. Diarrheal episodes separated by 3 symptom free days are considered as separate episodes. Diarrhea was defined as passing of more than 3 stools in 24 hours or stools with altered consistency, with or without mucus or blood or loose watery stools. Respiratory tract infections were defined as cough or cold with fever. If recurs after 3 symptom free days it is considered as a separate episode. Lower respiratory tract infections were confirmed by clinical examination and radiology.

Statistical analysis

There are two statistical traditions used for studying change. Both refer to this as growth curve analysis. It is important that growth can be positive or negative. One approach to studying growth curves is a structural equation modeling of latent growth curves and the other is called either hierarchical modeling or multi-level modeling (MLM).

Latent growth modeling is a statistical technique used in the structural equation modeling (SEM) framework to estimate growth trajectory. It is a longitudinal analysis technique to estimate growth over a period of time. It is widely used in the field of behavioural science, education and social science. It is also called latent growth curve analysis. SEM software such as AMOS (Analysis of Moment Structures), Mplus, LISREL (Linear Structural Relations), EQS (Equations), and R are used to estimate the growth trajectory. Latent growth model was derived from theories of SEM.

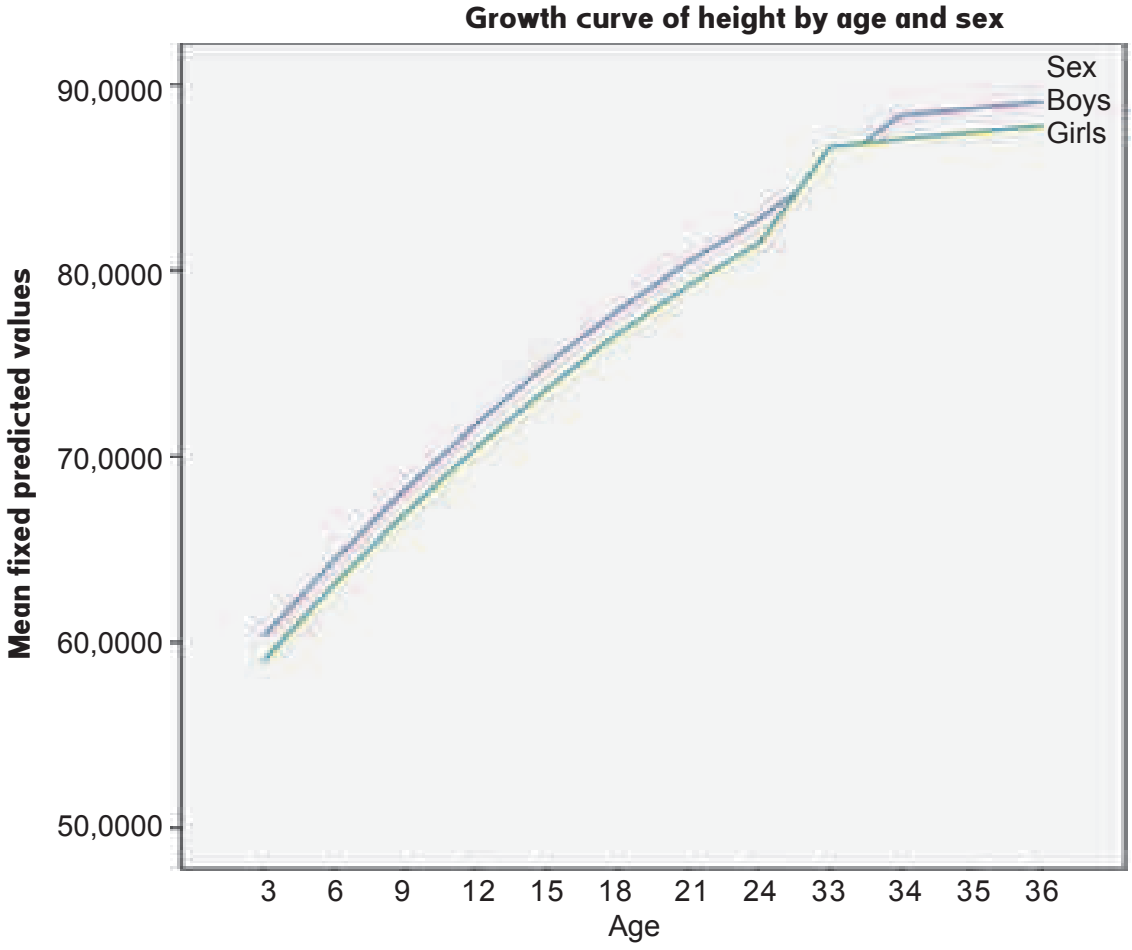
Latent Growth Models represent repeated measures of dependent variables as a function of time and other measures. The relative standing of an individual at a specific time point is modeled as a function of an underlying process, the parameter values of which vary randomly across individuals. Latent Growth Curve Methodology can be used to investigate systematic change, or

growth, and inter individual variability in this change. A special topic of interest is the correlation of the growth parameters, the so-called initial status and growth rate, as well as their relation with time varying and time invariant co-variates.

MLM is appropriate for data with a nested structure, such as repeated-measures in which several individual measurements (follow-ups) are nested within individuals (children). MLM allows the separation of the within-child (Level 1) and between-child variance (Level 2) in the outcome under study.

RESULTS

- Boys were significantly heavier (0.296g) and grew faster (1.12cm) than girls and on an average growth rates were observed for weight (0.400g) and height (1.51cm) and initial status of 3-36months children were 3.61kg and 55.8cm respectively. The shape of the weight and height curves are non linear.
- Growth rates were significantly higher in boys compare to girls for head (0.76cm),chest (0.84cm),mid upper arm (0.31cm) and calf circumferences (0.33cm). Average growth rates for head, chest, mid upper arm and calf circumferences are 0.79cm, 0.89cm, 0.26cm and 0.41cm.
- Percent body fat growth rates were similar in both gender and average growth trajectory was 0.10 and it shows non linear curve.
- The other co-variates are associated with height are gestational and maternal height and not maternal education and morbidity variables.



Estimates of Fixed Effects

Parameter	Estimate	Std. Error	df	t	Sig.	95% CILower	Upper Bound
Intercept	55.81	.921014	438.735	60.594	.000	53.998144	57.618442
[Male]	1.12	.263610	421.556	4.258	.000	.604409	1.640716
[Female]	0[a]	0	.	.	.	.	.
Illiterate	-.766	.937496	430.425	-.808	.419	-2.600391	1.084889
Primary	-.663	.942834	430.372	-.704	.482	-2.516418	1.189845
Secondary	-.602	.945729	430.580	-.636	.525	-2.460389	1.257252
College	0[a]	0	.	.	.	.	.
AGE	1.507072	.012413	2864.891	121.412	.000	1.482733	1.531412
Age2	-.016118	.000357	2866.318	-45.189	.000	-.016817	-.015419
G age (<40weeks)	-1.041373	.273891	421.756	-3.802	.000	-1.579734	-.503012
Gage_g>=40weeks	0[a]	0	.	.	.	.	.

4 TRAJECTORIES OF MATERNAL DEPRESSION, ANXIETY, GROWTH AND THEIR SOCIOECONOMIC DIFFERENTIALS

OBJECTIVE

The aim of the study is to identify trajectories for maternal depression, anxiety and infant growth and also study the relationships with co-variates of socio-demographic variables.

Background

Depression and anxiety disorders are the most common mental health problems among women. Research in developing countries indicates, maternal depression may be a risk factor for poor growth in young children. Women are particularly prone in the postpartum period because of hormonal changes occurring during this period and stressors associated with parenting The combined effect of women's vulnerability to depression, and their responsibility for child care could have a substantial influence on growth during early childhood.

Childhood growth is a key indicator of child health and nutritional status. According to recent estimates, India has a prevalence of 46% stunting and 47% under weight and 17% wasted (National Family Health Survey 2006). Inadequate growth during childhood is known to result in reduced adult stature, low educational performance, reduced economic productivity, impaired work capacity and heightened disease risk. Early childhood is important as there is rapid physical growth and development in early life when infants are dependent on the primary care giver for their

nutritional and needs, which makes young children vulnerable to the effects of their care givers' mental health problems. Recent research on the relationship between maternal depressive symptoms and child stunting or underweight has produced inconsistent results. According to a meta analysis by Surkanetal6 of the 17 studies on underweight and 5 of the 12 on stunting found a statistically significant relationship with maternal depression. However, all these studies have shown a negative association between maternal depression and infant growth.

Data available

The data to be used in this study come from the Indo-US collaborative study 'Efficacy of an Integrated Feeding and Care Intervention among 3 to 15 months old rural children in Andhra Pradesh India', which is a longitudinal study designed to investigate the role of infant feeding and care practices on infant growth and development outcomes from birth to 15 months of age (n=600). These are unique data with detailed depression and home observation measured environment data at 3 monthly intervals from 3-15 months, maternal knowledge, practice, and beliefs relating to feeding and child care, socio-demographic measures, maternal autonomy/ self esteem/ height/ weight, community environment measures, child development indicators at 9 and 15 months, monthly morbidity, height and weight of data. The study recruited all infants born across three mandals covered 60 villages in the rural Nalgonda district of Andhra Pradesh in India between Sep 2005-August 2006.

Statistical analysis

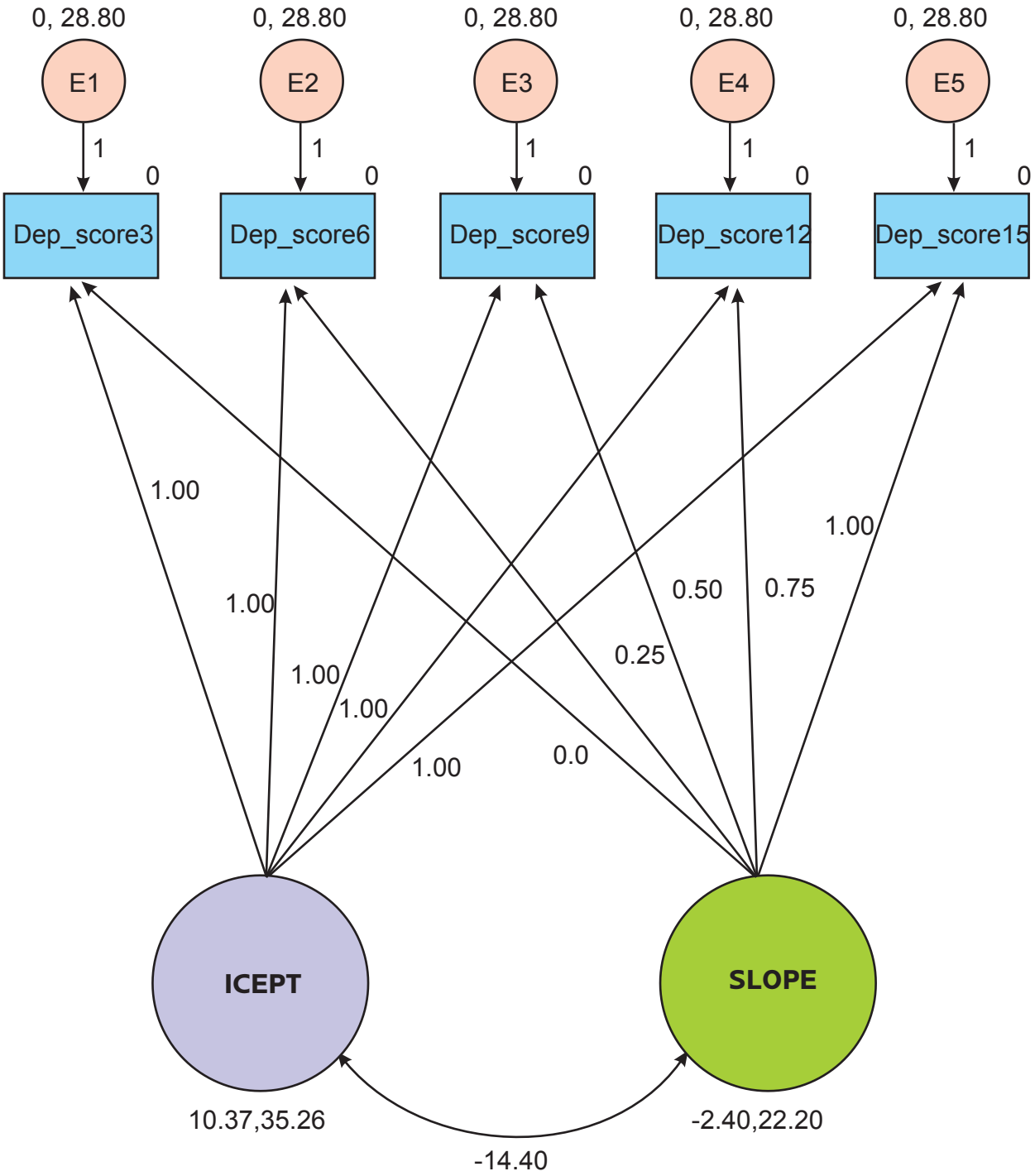
To accommodate the nested structure of the data and to obtain unbiased estimates, growth curve analysis of hierarchical linear modeling/Latent growth curve analysis will be performed. The growth curve models simultaneously estimate intra-individual time-based trajectories and test whether inter-individual differences in the parameters of these trajectories are a function of time-invariant and time-variant predictors.

RESULTS

The model is in a path diagram form in figure10. Understanding figure is a big step toward understanding LGC modeling. The variables in rectangular boxes are the only variables you observe, frustration among care givers. The y1 is the level of depression at 3 months, when the care givers start providing care; y2 is the same variable measured at 6months, perhaps after 6 months of providing care. The time 3 variable y3 is depression measured at the next time 9months, y4 is the depression level measured at 12 months and y5 is the fifth time (15-months). It is necessary to have the variable measured at least three times to estimate a linear trend. Five measurement points allow us to estimate a quadratic trend. At the bottom of Figure, the ɛi represent measurement errors because it is reasonable to assume that our measurement of depression is not perfectly reliable.

The intercept and slope are enclosed in ellipses because they are latent variables (not directly observed like our five measures of depression). The intercept (10.4) represents the initial level of depression. The slope (-2.4) represents the linear trend, whether depression decreases with time. The mean tells us the average mean and slope for the group. At the individual level, each person may have a different intercept and slope.

The overall model fit statistics is chi square =37.7, p =0.001. The RMSEA = .053, with a 90% confidence interval of .033 to .074. AMOS provides various model fit indices such as commonly used comparative fit (CFI) index, Tucker Lewis Index (TLI), and the normed fit index (NFI). The value of these indices for this model are good (CFI = 0.966, TLI = 0.963, and NFI = 0.946). In short, these statistics suggests an excellent fit of the model to the data. Initial/change of depression was not associated with maternal education but SLI was negatively related to SLI. Multi indicator Latent Curve is not a good fit.



The results show that the relation between maternal depression and infant growth (height) was inversely related even after adjusting covariates of possession of HH assets, child age and maternal education. No relationship was observed between weight of child and maternal depression.

III CLINICAL AND PHYSIOLOGICAL STUDIES

1 NUTRITIONAL CHALLENGES, ABDOMINAL ADIPOSITY AND TYPE 2 DIABETES IN INDIANS PARENTAL AND OFFSPRING CARDIO-METABOLIC RISK: A TRANS-GENERATIONAL EXTENSION OF HYDERABAD NUTRITION TRIAL. (ANDHRA PRADESH CHILDREN AND PARENTS STUDY-APCAPS)

Hyderabad Nutrition Trial (HNT) was conducted in Andhra Pradesh to assess the impact of food supplementation provided by Integrated Child Development Service (ICDS) during 1987-90. This supplementation was provided in 15 villages from Ranga Reddy District and 14 villages where the supplementation was not provided served as controls. Around 2000 women were recruited and randomized to serve as intervention group or controls to receive supplementation during pregnancy and the first five years of the child's life. There was an improvement in birth weight of about 61g in those infants whose mothers had received supplements (2655 ± 424g) compared with control infants (2594 ± 430g).

In the first follow up study from 2003-2005, these children (n=1165) were followed up during adolescence for anthropometric, biochemical and vascular parameters. The participants from the intervention villages were found to have more favorable measures of cardio-metabolic risk factors during their adolescence specifically with respect to insulin resistance and arterial stiffness: 20% (3% to 39%; P=0.02) lower HOMA score and 3.3% (1% to 5.7%; P=0.008) lower augmentation index (Kinra et al, BMJ, 2008). The study suggested that the improved maternal and child nutrition may have a role in reducing the burden of cardiovascular disease in low income and middle income countries. In the second follow up study (2008-10) these children were again recruited and were examined for various biochemical, anthropometric, physiological and vascular parameters along with the DEXA scans to evaluate the amount and distribution of body fat and differences between the various metabolic measures related to obesity, cardio-vascular diseases, diabetes and other chronic diseases among the HNT supplemented and non-supplemented children in their adult life.

In the present study named Andhra Pradesh Children and Parents Study (APCAPS), index children along with their the parents and siblings are also recruited to examine a wide range of risk factors relevant to obesity, diabetes and cardiovascular disease, thereby creating a trans-generation resource for future research.

Hypotheses

1. The parent-offspring effects on body mass index, blood pressure, lipid levels, diabetes and body composition in the Indian context may be different compared to data from developed countries.
2. The siblings of the index subjects who were born prior to the nutritional supplementation of the mothers may have a different cardio vascular risk profile as compared to those born during the supplementation period.

AIMS AND OBJECTIVES

1. To assess the inter-generational associations between genetic, biological and social exposures on obesity and cardio-metabolic diseases.
2. To assess the socio-biological determinants of the underlying distribution of chronic disease risk factors in a rural population.

3. To create a large scale three generation cohort resource including DNA, RNA, blood and detailed phenotyping relevant to cardio-metabolic diseases.
4. To see if children born before the nutritional supplementation of mothers differ from those born after, in their disease risk profile.

METHODOLOGY

The parents and siblings of the earlier study participants were invited to undergo the same examinations as in the previous study at our study clinics, and all the participants who have given consent without any chronic infections and diseases were included in the study. All pregnant women were excluded from the DEXA component of the study.

The Hyderabad DXA study has around 2600 subjects and, in this APCAPS it is proposed to recruit both the parents and elder sibling of the index subjects. Thus it was decided to invite around 8000 subjects from a total of 29 villages selected from 4 mandals in Ranga Reddy District. In addition to the parameters collected in the earlier study like anthropometry, Biochemical and physiological CV risk factors (questionnaires, anthropometry, DXA scan, arterial stiffness and carotid scan, and fasting bloods for glucose, lipids, insulin), saliva samples were collected for DNA analysis, as recent work suggests that it might give additional information related to epigenetic processes, for the inter-generational hypotheses to be examined.

Laboratory analysis

The blood samples were analyzed for fasting glucose in the clinical Division and complete blood picture in the Pathology department of National Institute of Nutrition (NIN), Hyderabad. Further, the serum samples are analyzed for Cholesterol, HDL, Trigylerides, Alkaline Phosphatase, Calcium, Phosphorous, Albumin, Creatinine, ALTL/SGPT, GGT, C-Reactive Protein at the coordinating centre (SANCD), New Delhi.

In addition, DNA is isolated from saliva and blood samples and stored for the proposed genetic analysis at SANCD, New Delhi.

RESULTS

Total 6944 participants from 1650 households in 29 villages were recruited till December 2012 at the village field clinic and carotid IMT measurements at NIN clinic were done for around 3861 subjects. In addition anthropometric data and birth details were collected in around 1250 preschool children in 13 villages. Data entry and analysis are in progress.

Preliminary analyses on 1403 individuals, aged 10-84 years, from 445 households, has shown the overall prevalence of self-reported chronic diseases in 39% of the households. Out of thirteen chronic diseases studied, hypertension (14%) was found to be the most prevalent followed by obesity (7.1%), peptic ulcer (6.3%), diabetes (5.8%) and asthma (4.6%). 13.5% of young individuals (19-39 years) were found to be overweight or obese (Body Mass Index$\geq$25kg/m^2) in the present study. Prevalence of cardio-metabolic risk factors is given in table below;

Prevalence of risk factors of cardio-metabolic diseases in adults by education

	Men [N-2028]			Women [N-1833]		
Risk Factors/conditions	Education Count (%)	No Education Count (%)	*p-value	Education Count (%)	No Education Count (%)	*p-value
N	1313 (64.74)	715 (35.26)		681 (37.15)	1152 (62.85)	
Overweight [BMI=25]	238 (18.13)	88 (12.31)	0.001	103 (15.12)	226 (19.62)	0.655
Underweight [BMI<18.5]	345 (26.28)	258 (36.08)	0.012	681 (34.95)	281 (24.39)	0.052

Prevalence of risk factors of cardio-metabolic diseases in adults by education (contd..)

	Men [N-2028]			Women [N-1833]		
Risk Factors/conditions	Education Count (%)	No Education Count (%)	*p-value	Education Count (%)	No Education Count (%)	*p-value
N	1313 (64.74)	715 (35.26)		681 (37.15)	1152 (62.85)	
High waist-hip ratio	275 (20.94)	284 (39.72)	0.123	105 (15.42)	397 (34.46)	0.107
Obesity [BMI=30]	28 (2.13)	13 (1.82)	0.402	29 (4.26)	42 (3.65)	0.080
Hypertension	112 (8.53)	137 (19.16)	0.056	35 (5.14)	149 (12.93)	0.444
Impaired fasting glucose	180 (13.71)	136 (19.02)	0.510	48 (7.05)	182 (15.80)	0.053
Diabetes [Glucose=126mg/d	35 (2.67)	24 (3.36)	0.012	43 (6.31)	61 (5.30)	0.072
Hypercholesterolemia	252 (19.19)	168 (23.50)	0.024	122 (17.91)	309 (26.82)	0.433
Hypertriglyceridaemia	383 (29.17)	231 (32.31)	0.337	119 (17.47)	262 (22.74)	0.567
Low HDL	536 (40.82)	207 (28.95)	0.024	416 (61.09)	713 (61.89)	0.533
High LDL [>3.5mmol/L]	174 (13.25)	106 (14.83)	0.087	95 (13.95)	228 (19.79)	0.102

High waist-hip ratio : Males: >0.90; Females>0.85; Hypertension: SBP≥140; DBP≥90;
Impaired glucose tolerance : fasting glucose >=100 & <126;
Hypercholesterolemia : >5.2mmol/L; Low HDL: Male<1mmol/L, Female<1.3mmol/L;
Hypertriglyceridaemia : >1.7mmol/L;

Prevalence of risk factors of cardio-metabolic diseases in adults by occupation

	Men [N-2028]			Women [N-1833]		
Risk Factors/conditions	Skilled Count (%)	Unskilled Count (%)	*p-value	Skilled Count (%)	Unskilled Count (%)	*p-value
N	813 (40.00)	871 (42.94)		206 (11.23)	999 (54.50)	
Underweight [BMI<18.5]	172 (21.16)	300 (34.44)	0.000	62 (30.10)	273 (27.33)	0.917
Overweight [BMI=25]	187 (23.00)	110 (12.63)	0.000	37 (17.96)	167 (16.72)	0.281
Obesity [BMI=30]	27 (3.32)	9 (1.03)	0.000	8 (3.88)	22 (2.20)	0.234
High waist-hip ratio	243 (29.89)	292 (33.52)	0.000	46 (22.33)	305 (30.53)	0.499
Hypertension	102 (12.55)	127 (14.58)	0.000	10 (4.85)	116 (11.61)	0.157
Impaired fasting glucose	125 (15.38)	146 (16.76)	0.318	29 (14.08)	132 (13.21)	0.136
Diabetes [Glucose= 126mg/dl]	23 (2.83)	32 (3.67)	0.526	11 (5.34)	48 (4.80)	0.518
High insulin [insulin > 60pmol]	292 (35.92)	142 (16.30)	0.000	67 (32.52)	212 (21.22)	0.002
Hypercholesterolemia	202 (24.85)	185 (21.24)	0.000	50 (24.270	231 (23.12)	0.046
High LDL [>3.5mmol/L]	135 (16.61)	117 (13.43)	0.000	36 (17.48)	171 (17.12)	0.146
Low HDL	339 (41.70)	276 (31.69)	0.051	123 (59.71)	616 (61.66)	0.586
Hypertriglyceridaemia	296 (36.41)	263 (30.20)	0.000	29 (14.08)	222 (22.22)	0.137

Impaired glucose tolerance: fasting glucose >=100 & <126; High WHR: Males: >0.90; Females>0.85;
Hypertension: SBP≥140; DBP≥90; Low HDL: Male<1mmol/L, Female<1.3mmol/L;
Hypertriglyceridaemia: >1.7mmol/L; Hypercholesterolemia: >5.2mmol/L; ^

IV MICROBIOLOGY AND IMMUNOLOGY

1 ANTICARCINOGENIC PROPERTY OF PROBIOTIC IN COMBINATION WITH *ALLIUM SATIVUM* AND NSAIDS ON DMH INDUCED COLON CANCER AND COLON CANCER STEM CELLS IN RATS

Epidemiological studies and animal experiments suggest reduced risk of cancer and other chronic conditions with certain foods and dietary constituents. Food consists of a complex mixture of a wide variety of components, many of which are biologically active. Colorectal cancer (CRC) is one of the most common cancers world-wide, with highest incidence rates in western countries. In recent years, much effort has been dedicated in search for natural or pharmacological preventive agents, which would block or attenuate CRC process. In addition to having anti-inflammatory properties, non-steroidal anti-inflammatory drugs (NSAIDs) inhibit neoplastic cell proliferation by inducing apoptosis. Inhibition of cyclooxygenase-2 (COX-2) seemed to be the principal target of NSAIDs, as it is overexpressed in several cancers and catalyzes the synthesis of prostaglandin E2 (PGE2), the critical pro-inflammatory molecule. A major role for phosphatidylinositol-3 kinase (PI3-kinase) pathway activation in human tumors has been more recently established. The present study explored the role of PI3- kinase and Wnt molecular pathways in COX-2 and PGE2 production as well as NSAIDs' combination with garlic and *Lactobacillus rhamnosus*, chemopreventive effect in colon cancer. 1, 2-dimethylhydrazine (DMH) was used for experimental colon cancer model in rat. Long term use of NSAIDs leads to development of ulcers and inflammation and also reduces blood platelets count. *Lactobacillus rhamnosus GG (*LGG) and *Allium Sativum* taken as adjuvant may increase the efficacy and potential of NSAIDs and decrease the side effects.

METHODOLOGY

- Wistar rats male (100-120g) used for this study were sourced and housed at the National Centre for Laboratory Animal Sciences, Hyderabad, India and kept in plastic cages with saw dust (renewed after every 48 h), under a controlled temperature of 23–25^{0}C and 12 h light–dark cycle. Animals were given AIN 76 diet. Animals had free access to food and water *ad libitum* throughout the study.
- Colon cancer was be induced with the carcinogen 1, 2 dimethylhydrazine dihydrochloride. Each rat of all groups except group I will receive subcutaneously, 30 mg DMH/ Kg body weight weekly during 6 weeks
- Preparation of Lyophilized garlic powder by SCANVAC Cool safe™
- Garlic powder (5% of the diet)
- NSAIDs (Aspirin) (50mg/ kg body weight)
- Probiotic preparations: lyophilized *Lactobacillus rhamnosus* G (~10^8cfu/g of diet).
- Vsl #3 (Probiotic cocktail) 112 billion bacteria per capsule single capsule per day
- Identification of preneoplastic biomarker for ACF colon samples will stained with 0.2% methylene blue for 5 min, and the mucosal side will placed on a glass slide and examined microscopically using ×10 objective for assessment of the number of aberrant crypts (AC) following a procedure described by Bird.
- MDF Marker in colon tissue. After ACF determination, MB-stained colons will be kept in formalin and then processed with the high-iron diamine Alcian blue staining (HID-AB) to visualize MDF.

The HID-AB-stained colons will be scored at the microscope (40 × magnification) and MDF identified as focal lesions characterized by the absence or very limited production of mucins.

- Histopathologial analysis of colon tissue and tumors
- Scan electron microscopy of colon and intestine.
- Specific Primer were used to determine the expression of different gene (Cyclin D1, bax, bcl2, APC, cox, Wnt etc)
- Expression of oncogene, tumour suppressor gene by RT PCR
- DNA fingerprinting of fecal and cecal lactobacilli of each groups was carried out by using RAPD-PCR technique
- Blood samples of each group of animal were analyzed for serum inflammatory and anti-inflammatory marker TNF-α, IL-10 and INF-γ
- Blood picture analysis.

Microarray Target Preparation and Hybridization. Affymetrix A Rat 1.1 ST array were used in this experiment. For total RNA isolation, the colonic mucosa was homogenized using a homogenizer in TRIzol reagent. After RNA extraction, samples were cleaned with minicolumns, quantified using a UV spectrophotometer (*A*260/*A*280), and the quality of RNA assessed by agarose-formaldehyde gel electrophoresis. Only high quality RNA was used in subsequent steps.

Experimental design for animal studies

- To study the objectives, rats will be divided into 11 groups with 18 rats in each group

Group	Composition
GP1	DMH+CTL
GP2	DMH+GARLIC
GP3	DMH+LGG
GP4	DMH+VSL 3
GP5	DMH+ASPRIN +GARLIC

Group	Composition
GP6	DMH+ASPRIN+LGG
GP7	DMH+ASPRIN+ LGG+GARLIC
GP8	DMH+GARLIC+LGG
GP9	DMH+ASPRIN
ASPRIN CONTROL	ASPRIN CTL
NORMAL CONTROL	NORMAL CTL

The following analysis was performed,

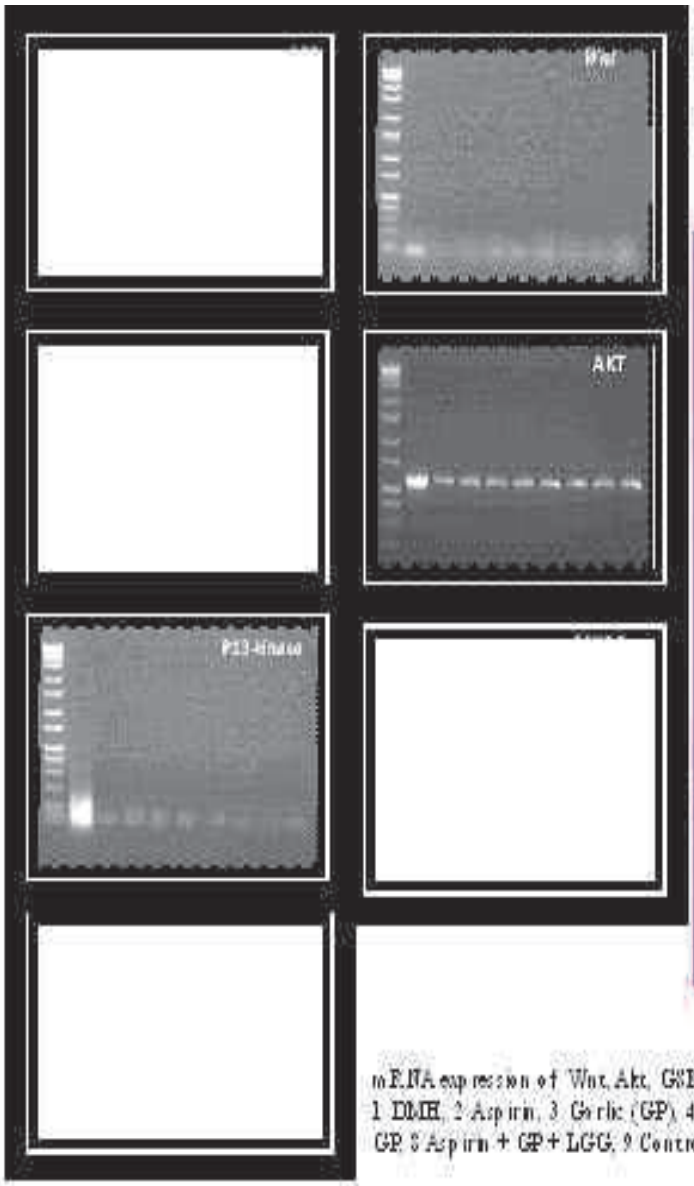
- Data quantification and normalization
- Identification of differentially expressed genes
- Annotation and GO analysis of the differentially expressed genes/transcripts
- Pathway enrichment

RESULTS

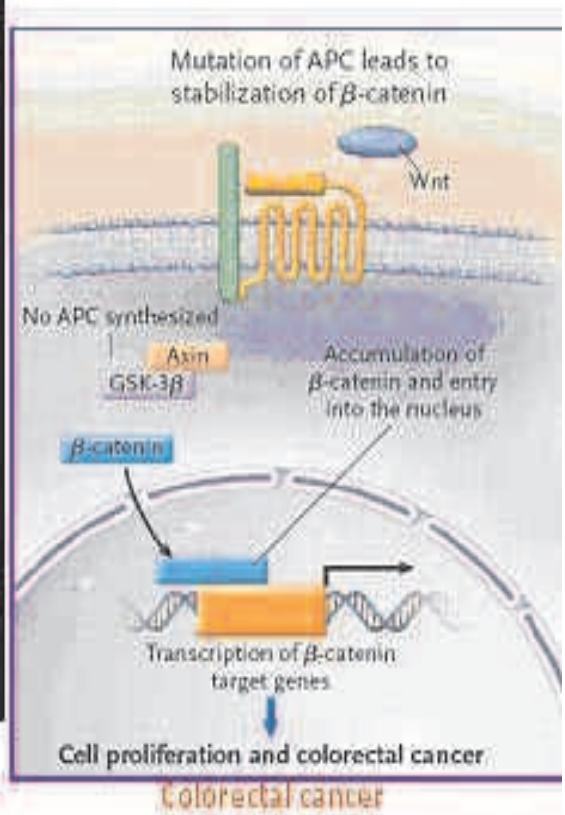
The incidence and number of tumors in the colon were significantly higher when the rats were administered DMH, as compared to treatment groups. The incidence of aberrant crypts (AC) formation induced by DMH was reduced to 84.1% and histopathological alteration was also prevented in rats supplemented with NSAIDs with garlic and *Lactobacillus rhamnosus* GG. As Wnt/β-catenin signaling is an essential determinant of colonic cellular fate and is found disrupted in adenocarcinoma, we examined its role in the chemoprevention of experimental colon cancer by NSAIDs, *Lactobacillus rhamnosus GG (*LGG), *Allium Sativum*). Treatment of rats with a colon-

specific carcinogen, DMH, increased the Wnt and β-catenin levels significantly. However, NSAIDs reduced the levels of Wnt and β-catenin appreciably when administered simultaneously with DMH. There was a significant increase in β-catenin expression after DMH treatment. Control as well as probiotic combination groups expressed a low level of Wnt and β-catenin. COX-2 mRNA expression as well as PGE2 levels was elevated after DMH treatment; however, COX-1 mRNA expression was unaltered as seen by reverse transcriptase-polymerase chain reaction (RTPCR) analysis. DMH also activated PI3-kinase, Akt, Wnt, and β-catenin expressions but reduced the glycogen synthase kinase-3β (GSK-3β) levels.

In this study, microarray experiment was performed on twelve Rat samples using the Affymetrix technology (Affymetrix Rat gene 1.1 ST array plate) to identify the differentially expressed genes/transcripts. Globally scaled data were then imported into GeneSpring (Silicon Genetics, Inc., Redwood City, CA, Version 6.0) for normalization and filtering. Microarray experiment demonstrated that PI3-kinase/Akt and Wnt/β-catenin pathway mediates key signals for intestinal epithelial cell proliferation and inhibition of apoptosis in an experimental model of colon cancer.



mRNA expression of PI3-kinase and Wnt signaling genes as seen by RT-PCR in the colonic mucosa of rat.



mRNA expression of Wnt, Akt, GSK3β as seen by RT-PCR in the colonic mucosa of rat. 1 DMH, 2 Aspirin, 3 Garlic (GP), 4 LGG + GP, 5 LGG + GP, 6 Aspirin + LGG, 7Aspirin + GP, 8 Aspirin + GP + LGG, 9 Control

Table : Selected genes significantly affected by carcinogen (DMH) treatment in the colonic epithelium of male wistar rats

Gene Symbol	Gene title	DMH (fold change)
(I) Extracellular matrix, cell adhesion, cytoskeleton		
Fn1	Fibronectin 1	1.7
Col1a1	Collagen, type I, alpha 1	
Vim	Vimentin	1.5
Tmsb10	Thymosin, beta 10	1.3
Col3a1	Collagen, type III, alpha	1.3
(II) Immune, defense, inflammation, stress		
Pla2g2a	Phospholipase A2, group IIA (platelets, synovial fluid)	2.4
Cd74	Cd74 molecule, major histocompatibility complex, class II invariant chain	2.1
Lyz2	lysozyme 2	2.1
RT1-Db1	RT1 class II, locus Db1	2.0
RT1-DMb	RT1 class II, locus DMb	1.7
RatNP-3	Defensin RatNP-3 precursor	1.7
RT1-Da	Histocompatibility 2, class II antigen E alpha	1.6
RT1-Ba	RT1 class II, locus Ba	1.5
Cxcl13	Chemokine (C-X-C motif) ligand 13	1.5
RT1-Bb	RT1 class II, locus Bb	1.5
Ccl2	Chemokine (C-C motif) ligand 2	1.4
Mif	Macrophage migration inhibitory factor	1.3
RT1-M3-1	RT1 class Ib, locus M3, gene 1	1.3
Irf7	Interferon regulatory factor 7	1.3
Cxcr4	Chemokine (C-X-C motif) receptor 4	1.3
Il15	Interleukin 15	0.6
(III) Protein processing, synthesis, degradation		
Rps7	Ribosomal protein S7	1.4
Rps15	Ribosomal protein S15	1.4
Pfdn2	Prefoldin subunit 2	1.4
Hspe1	Heat shock protein 1 (chaperonin 10)	1.3
Rpl37	Ribosomal protein L37	1.3
Rpl4	Ribosomal protein L4	1.3
Psmb4	Proteasome (prosome,macropain) subunit, beta type 4	1.3
Rpl3l	Ribosomal protein l3-like	1.3
Rps4x	Ribosomal protein S4, x-linked	1.3
Rps17	Ribosomal protein S17	1.3
Rpl36al	Ribosomal protein l36a-like	1.3
Rps9	Ribosomal protein S9	1.3

Data expressed as mean-fold change normalized to saline-injected animals. *All genes presented were significantly altered compared to saline injected* animals (*P < 0.05).*

CONCLUSIONS

- Treatment with Allium Sativum, Lactobacillus rhamnosus GG and NSAIDs prevented DMH induced histopathological alterations and tumour formation in the colonic tissue; and inhibited Wnt and β-catenin expression.
- Microarray experiment demonstrated that PI3-kinase/Akt and Wnt/β-catenin pathway mediates key signals for intestinal epithelial cell proliferation and inhibition of apoptosis in an experimental model of colon cancer.
- Inhibition of Wnt and β-catenin pathways and induction of GSK-3β, which may eventually activate apoptosis and block key signals for intestinal epithelial cell proliferation appear to be the potential molecular pathway.

2 METABOLIC ENDOTOXEMIA AND ASSOCIATED METABOLIC DISORDERS IN RATS FED DIFFERENT DIETS AND THEIR RELATION TO SELECTIVE GUT BACTERIA AND CHANGES IN INTESTINAL PERMEABILITY

In India, a large proportion of population is insulin resistant and the prevalence of diabetes and CHD is high. Quantity and quality of diet has lot of implications in causing diabetes and CHD. Research findings also reveal that different fatty acids have different effects on insulin action. A low-fat diet didn't really prevent type 2 diabetes when compared to a low-carb diet. Low-carb diets thwart diabetes better than low-fat diets. These results are a bit surprising as most doctors and nutritionists suggest a low-fat diet to prevent type 2 diabetes. The microbiota of the human intestinal tract play an important role in health, in particular by mediating many of the effects of diet upon gut health. At the interface between the luminal content and host tissues, the intestinal epithelium must integrate pro- and anti-inflammatory signals to regulate innate and adaptive immune responses, i.e. to control inflammation. However, under the influence of environmental factors, disturbance of the dialog between enteric bacteria and epithelial cells contributes to the development of chronic inflammation and associated disorders.

Diabetes and obesity are characterized by a low-grade inflammation. Many diseases including obesity, cardiovascular disease, diabetes (Type 2), associated with metabolic complications could arise from imbalances of microflora in the gastro-intestinal tract. However studies are lacking to provide a clinical correlation between resident bacterial flora of the gut and the metabolic complications. It is believed that gut flora in Indians differs from that of westerns. As in India, a large proportion of population is insulin resistant and the prevalence of diabetes and CHD is high, it is pertinent to understand the effect of different diets (as per Indian pattern) on the gut microbiota in terms of metabolic endotoxemia and its associated metabolic complications and the mechanism involved in alteration of intestinal permeability brought about by diet through gut flora.

AIMS AND OBJECTIVES

1) To assess, under controlled conditions, the impact of various cooking oils in rats fed isocaloric diet or high fat (HF) or high carbohydrate (HC) diets on selective gut bacteria (Bifidobacterium,

Lactobacillus, Bacteroides) in relation to metabolic endotoxemia and its associated metabolic complications.

(2) To study the alteration in intestinal permeability in these rats by measuring intestinal acidic sphingomyelinase enzyme activity and correlate with gut bacteria, metabolic endotoxemia, plasma -- glucose, insulin and lipid profile.

Part 1: Effects of different oils used in an isocaloric diet on metabolic disorders in relation to selective gut bacterial alterations (Work done during 2010-2011)

Plan of work:

Sprague Dawley rats were fed an isocaloric diet varying only in the quality of fat as per Table I. The effect of feeding flax seed powder was tested only in rats given groundnut oil or vanaspathi.

METHODOLOGY

Studies were carried out in rats to test the effect of an isocaloric diet (Table 1) differing only in the quality of fat (different cooking oils like grountnut oil, sun flower oil, palm oil, ghee and vanaspathi) on gut flora and its association with intestinal permeability and plasma endotoxin, biochemical profile and body composition. Detailed methodology was already given in the last year's Annual Report (2010-11).

RESULTS

Lactobacilli were significantly lower in palm oil fed group compared to other groups (Table 2). The beneficial bacteria, Bifidobacteria levels were remarkably lower in vanaspathi fed group and this decrease was statistically significant from Sun flower oil fed group which had the highest bifidobacterial count. Bacteroides were significantly higher in sunflower oil, ghee and vanaspathi fed groups compared to groundnut and palm oil fed groups (Table 2). No changes were observed in acidic sphingomyelinase activity and reactive oxygen species in the intestine and in line with this observation there were no changes in plasma endotoxin levels in different groups. Glucose, Insulin, HOMA index were significantly higher in vanaspathi fed group compared to other groups (Table 3). Among the 3 saturated fat oil fed groups Fat % was significantly higher and lean body mass and fat free mass were significantly lower in ghee and vanaspathi fed groups compared to palm oil fed group. Bone Mineral Density was significantly higher and Lean body mass and fat free mass were significantly higher in palm oil fed group compared to groundnut and sunflower oil fed groups. It can be concluded that the changes seen in biochemical profile or body composition are not mediated through plasma endotoxin although there were changes in bacterial profile. Feeding flax seed powder to hydrogenated fat (vanaspathi) fed rats significantly reduced total cholesterol and triglycerides (Table 4).

Table 1. Isocaloric Diet Composition (gm / 100gm Diet)

SD rats groups (n=7)	Starch	Sucrose	Casein	Fat*	Cellulose	Mineral mixture	Vitamin mixture	L-Cysteine	Choline chloride
I	59.5	0	20	10 GR	5	4	1	0.3	0.2
II	29.75	29.75	20	10 GR	5	4	1	0.3	0.2
III	29.75	29.75	20	10 SU				0.3	0.2
IV	29.75	29.75	20	10 PA	5	4	1	0.3	0.2
V	29.75	29.75	20	6 GH+ 4SU	5	4	1	0.3	0.2
VI	29.75	29.75	20	6 VA+4 SU	5	4	1	0.3	0.2
VII	24.75	24.75	20	10 GR+10FL	5	4	1	0.3	0.2
VIII	24.75	24.75	20	6VA+4SU+10FL	5	4	1	0.3	0.2
IX	Cereal based diet								

Group I: groundnut oil (GR); group II: groundnut oil+sucrose; group III: sunflower oil(SU), group IV : PA = palmolein, group V: ghee(GH)+SU; group VI: vanaspathi (VA) + SU; group VII: GR+flax seed (FL) powder; group VIII: VA+SU+FL

Table 2. Effect of feeding diets varying only in the quality of oil in an isocaloric diet on ceacal bacteria (CFU/gm)

Bacteria	II (6)GR	III (7)SU	IV (4)PA	V (7)GH	VI (5)VA
Lactobacilli $X10^6$	227.8± 15.5[a]	191.36± 32.4 [a]	93.3±38. 6 [b]	155.07±28.9 [a]	169.8± 45.6 [a]
Bifidobac $x10^6$	116.75± 11.9	168.29± 19.3 [b]	126.5± 58.4	169.79± 48.2[b]	69.2± 21.0[a]
Bacteroides $x10^4$	36.08± 16.5 [a]	107.5 ± 43.2 [b]	18.8 ±8.98 [a]	142.93 ± 42.4 [b]	120.7± 26.1 [b]
Enterobacteriace a $x10^4$	184.08±42.5 [a]	206.57± 43.4 [a]	167.5± 61.8 [a]	105.5± 51 [a]	143.2± 65.4 [a]
Streptococcix 10^4	13.9± 7.42 [a]	32.4± 10.6 [a]	9.5± 2.35 [a]	67.1± 11.2[b]	88.2± 38.1 [b]
Enterococci $x10^6$	145.5±39. 5 [a]	100.86± 34.6 [a]	95.8± 41.1 [a]	101.86± 42.4 [a]	61.2± 29.5 [a]
Endotoxin ng/ml	95.5±11.1 [a] (6)	100.50±6.89 [a] (5)	96.500±9.50 [a] (3)	89.100±8.47 [a] (5)	112.13±11.3 [a] (4)

Values are mean±SE. Values bearing even one common superscript are not statistically significantly at P<0.05. Values in parenthesis is sample size.

Table 3. Effect of feeding diets varying only in the quality of oil in an isocaloric diet on body composition and some biochemical parameters

Parameters	II GR	III SU	IV PA	V GH	VI VA
Food intake gms	2137±84.3 [a] (7)	2138±59.6[a] (7)	2541.25± 39.53 [b] (4)	2373±47.1 [b] (7)	2186±134.59 [a] (6)
Body weight gms	392.00±13.2 [bc] (7)	387.00±11.0 [cd] (7)	417.25±10.2[bc] (4)	416.71±11.1[ab] (7)	394.40±20.8[bc] (7)
Glucose mg/dl	78.9±9.44 [a] (7)	72.0±2.30 [a] (7)	87.4±6.41 [a] (5)	82.8±9.00 [a] (5)	89.2±5.70 [a] (5)
Insulin munits.L	6.80±1.05[a] (7)	7.62±0.96 [a] (5)	9.00±1.29 [a] (4)	6.67±0.58 [a] (3)	12.3±1.86[b] (5)
HOMA	1.27±0.20 [a] (7)	1.39±0.22 [a] (5)	1.79±0.38 [a] (3)	1.15±0.10 [a] (3)	2.32±0.35 [b] (4)
Cholesterol mg/dl	65.9±2.95 [a] (7)	55.4±1.89 [bc] (7)	68.7±1.38 [a] (4)	59.8±3.168 [ac] (5)	61.6±5.97 [ac] (5)
Triglyceride mg/dl	59.0±2.61 [a] (7)	59.1±2.668 [a] (7)	66.0±7.691 [a] (4)	79.5±4.41[b] (4)	66.0±4.654 [a] (4)
Fat%	22.9±0.48 (7)	22.7±0.90 (7)	22.3±0.68 (4)	24.3±1.00 (7)	24.2±0.66 (7)
BMD gms	0.136±0.001[a] (7)	0.141±0.001[ac] (7)	0.145±0.0009[bc] (4)	0.145±0.0015[bc] (7)	0.142±0.003[bc] (7)
LBM gms	280.92±7.24[a] (7)	272.90±7.03[a] (7)	311.16±2.73[bc] (4)	283.25±10.2[a] (7)	286.92±10.2[ac] (7)
FFM gms	192.56±4.17 [ac] (7)	187.44±5.19 [c] (7)	211.25±4.90 [ab] (5)	199.62±8.03 [ac] (7)	194.33±8.20 [ac] (7)
SMase U/mt/mgpr	557.99±82.8 [ac] (5)	865.79±243.12 [a] (5)	648.00±64.6 [ac] (4)	786.07±158.65 [a] (7)	318.62±69.9 [bc] (5)
H_20_2 mmoles/mt/mg protein	2.95±0.105a (4)	3.20±0.233 a (5)	3.13±0.514 a (4)	4.71±0.449 b (7)	3.24±0.496 a (4)

Table 4. Effect of flax seed powder on Food intake, Triglyceride and Cholesterol in groundnut oil or hydrogenated fat fed rats

	II vs VIII		VI vs VII	
	GR	GR+FLAX	VA	VA+FLAX
Food intake gms	2137 84.3 [a] (7)	2265 59.3 [a] (6)	2187 134.59 [a] (6)	2369 40.8 [a] (6)
Triglyceride mg/dl	59.0 2.61 [a] (7)	59.5 3.81 [a] (6)	73.8 8.59 [a] (4)	51.2 2.27 [b] (6)
Cholesterol mg/dl	65.9 2.95 [a] (7)	56.0 3.15 [b] (6)	61.6 5.97 [a] (5)	51.2 1.08 [a] (6)

Values are mean±SE. Values bearing even one common superscript are not statistically significantly at P<0.05. Value in parenthesis is sample size

CONCLUSIONS

- Rats fed hydrogenated fat (vanaspathi) had higher body weight, higher insulin resistance, higher body fat %, lower Lean body mass (LBM), lower fat free mass (FFM) and lower bone mineral density (BMD) and had lower bifidobacterial count and higher bacteroidetes.
- There were no changes in plasma endotoxin levels, intestinal acidic sphingomyelinase (ASmase) and reactive oxygen species suggesting intact intestinal membrane integrity.
- Feeding flax seed powder to hydrogenated (vanaspathi) fat fed rats significantly reduced total cholesterol and triglycerides.

This study shows that there is differential gut bacterial alteration with the quality of oil used in the isocaloric diet. In rats fed hydrogenated fat there were alterations in gut bacteria in association with adverse effects on body composition, insulin sensitivity. However, endotoxin levels, ASmase and reactive oxygen species were normal suggesting intact intestinal integrity that signifies other mechanisms underlying the adverse changes observed in body composition and insulin sensitivity.

Part 2: Effects of high fat /high carbohydrate diets on metabolic disorders in relation to selective gut bacterial alterations

In India a large proportion of population is insulin resistant and the prevalence of diabetes and CHD is high. The link between high fat (HF) diet and insulin resistance is well known. Evidence suggests that HF diet and metabolic disorders (type 2 diabetes and insulin resistance) are tightly linked to inflammation. However the exact cause of increased prevalence of diabetes in Indians is not known. Indian population is known to consume more of high Carbohydrate (HC) diet rather than HF diet.

Cereals are staple diet in India, and carbohydrate consumption averages to 60% of the bulk of the total calorie intake. Studies reported in the literature show that high carbohydrate consumption also results in an increase in plasma glucose, insulin, triglycerides and non-esterified fatty acids leading to insulin resistance. It has been hypothesized that the higher rates of CVD and type 2 diabetes mellitus in India after 1960s-70s could be due to the consumption of carbohydrates which undergo a high degree of refining and milling (8-12%) resulting in the loss of dietary fibre and various micronutrients.

In general, much of the work linking food and metabolic disorders (type 2 diabetes and insulin resistance) have been focused on studying food fat content and less have been concentrated on the importance of carbohydrate composition. These studies have been on animal models where the fat used is of animal origin (lard) and intakes range from 40 to 75% of total kilocalories.

Another fat similar to Lard in its fatty acid profile is palmolein which is of plant origin and which is widely used in the fast foods and bakery products and also in households in India for its good taste and affordability. It is not surprising to see that childhood obesity is on the rise as the fast foods and bakery products are the preferred food choices of children nowadays. However, there is a long standing controversy on whether palmolein is really beneficial and more so at high doses than the recommended intakes of dietary fat. Also, studies reported on the effects of palmolein on lipids and other biochemical parameters are controversial and not many studies are there on the long term usage of palmolein. There are reports to show that when a portion of the fat from a high polyunsaturated fat diet (60% total kcal as fat) was replaced with fish oil (6% kcal of total kcal) containing long-chain n-3 fatty acids, glucose uptake in skeletal muscle and hepatic glucose output were restored to levels observed in chow-fed rats. In this context omega-3 fatty acids, usually from fish oil, have been shown to reduce inflammation and help prevent certain chronic diseases, such as heart disease and arthritis. However, flax seed oil, which is rich in omega 3 fatty acid, has limited scientific evidence.

The microbiota of the human intestinal tract plays an important role in health, in particular by mediating many of the effects of diet upon gut health. Under the influence of environmental factors, disturbance of the dialog between enteric bacteria and epithelial cells contributes to the development of chronic inflammation and associated disorders. Diabetes and obesity are characterized by a low-grade inflammation. There are studies to show that the fecal microbiota in clinical conditions like diarrhea, inflammatory bowel disease, irritable bowel syndrome, atopic disorders differs significantly from that of healthy subjects.

However, studies are lacking to provide a clinical correlation between resident bacterial flora of the gut and the metabolic complications associated with obesity. Hitherto there is one recent evidence suggesting that a particular gut microbial community may favour occurrence of the metabolic diseases. Moreover, to the best of our knowledge there are no studies reported on the effects of cooking oils which are commonly consumed in India on gut microbiota. Keeping in view all the above facts, the present study was designed to study the effects of HF / HC diet, with palmolein oil as the source of high fat and flax seed oil was supplemented to test if it can reverse the effects seen with HF diets on selective gut bacteria, plasma biochemical parameters, body composition, immune function and inflammation in rats.

AIMS AND OBJECTIVES

1) To assess under controlled conditions, the impact of a vegetable based palmitic acid rich high fat diet or a high starch diet on various metabolic parameters in relation to selective gut bacterial alterations in Wistar /NIN rats.

2) To see the effects of flax seed oil in rats fed high palmolein diet.

This study was designed to study the effects of high fat or high carbohydrate diets, with palmolein as the source of high fat (HF) (53 energy%) and corn starch (78 energy %) as the source of high carbohydrate (HC) comparing with a control group (CT) fed groundnut oil at 22 energy% and starch at 59 energy% in the diet on selective gut bacteria, plasma biochemical parameters, body composition, immune function and inflammation in rats. Also, flax seed oil was supplemented at two different concentrations to test its influence on HF diet fed rats.

METHODOLOGY

The methodology used for studying the various parameters was already given in the annual report (2010-11).

RESULTS

The results obtained are given in the following tables (Tables 2 - 6)

Table 1. High fat/ high carbohydrate diet composition (gm/ 100gm diet)*

Groups	Starch	Sucrose	Total Calories from Carbohydrate	Casein	Fat
CT (408)	60 (58.8%)	0	(58.8%)	19.5 (19%)	10 Groundnut (22%)
HF (508)	19 (15%)	19 (15%)	(30%)	21.5 (16.9%)	30 Palmolein (53.1%)
HFF1 (508)	19 (15%)	19 (15%)	(30%)	21.5 (16.9%)	30 Palmolein (53.1%) + Flax oil 1
HFF2 (508)	19 (15%)	19 (15%)	(30%)	21.5 (16.9%)	30 Palmolein (53.1%)+ Flax oil 2
HC (383)	55.9 (58.4%)	18.6 (19.4%)	(77.8%)	10 (10.44%)	5 Palmolein (11.7%)

*Cellulose-5%, Salt Mix – 4%,Vitamin Mix—1%, L-Cysteine– 0.3%, Choline Chloride--0.2%
Values in parentheses are calories Flax oil 1 and Flax oil 2 : 7.5ml or 15ml /kg body weight respectively.

Table 2. Effect of high fat/ high carbohydrate feeding on energy intakes and body weight – influence of flax seed oil

	CT	HF	HFF1	HFF2	HC
Initial body weight (gm) P value	60.6±6.70 (7)	60.3±6.57 (7) 0.976	60.4±6.51 (7) 0.988	60.0±6.35 (7) 0.951	59.7±5.86 (7) 0.925
Final body weight (gm) P value	325.20±10.2 (5)	316.67±19.1 (6) 0.705	320.33±12.9 (6) 0.829	305.86±14.6 (7) 0.378	303.00±14.8 (5) 0.250
Food intake (gm/week) P value	91.1±5.24 (8)	61.9±3.25 (8) 0.001	54.7±1.22 (8) 0.001	50.9±1.58 (8) 0.001	93.3±6.26 (7) 0.698
Total Energy Intake (cals /week) P value	371.47±21.4 (8)	277.60±14.6 (8) 0.001	303.17±10.9 (8) 0.010	344.02±18.0 (8) 0.284	357.13±23.9 (7) 0.586
Gain in body weight (gm/week) P value	26.7±1.74 (8)	24.2±1.60 (8) 0.277	23.5±1.32 (8) 0.172	22.3±1.65 (8) 0.063	18.7±1.81 (7) 0.002
Feed Conversion ratio	14.2±0.97 (8)	11.9±1.19 (8) 0.260	13.2±0.91 (8) 0.621	16.3±1.69 (8) 0.298	19.9±2.08 (7) 0.007
Feed efficiency P value	0.073±0.005 (8)	0.089±0.008 (8) 0.081	0.078±0.005 (8) 0.540	0.067±0.008 (8) 0.540	0.053±0.005 (7) 0.045
E – CHO (cals /week) P value	218.51±12.6 (8)	57.0±2.99 (8) 0.001	50.3±1.13 (8) 0.001	46.8±1.45 (8) 0.001	277.87±18.7 (7) 0.001
E – FAT (cals /week) P value	81.9±4.71 (8)	167.30±8.77 (8) 0.001	205.78±9.08 (8) 0.001	253.47±16.0 (8) 0.001	42.0±2.82 (7) 0.007
E-PRO (cals/week) P value	71.0±4.08 (8)	53.3±2.79 (8) 0.001	47.1±1.05 (8) 0.001	43.8±1.36 (8) 0.001	37.3±2.50 (7) 0.001

Table 3. Effect of high fat /high carbohydrate feeding on immune and inflammatory parameters - influence of flax seed oil

	CT	HF	HFF1	HFF2	HC
Neutrophils (%) P Value	23.7±3.19 (6)	33.6±5.42 (8) 0.061	21.9±1.78 (7) 0.733	22.0±1.55 (8) 0.746	21.4±2.02 (7) 0.554
WBC (10^3/mm3) P Value	11.0±0.93 (5)	8.36±1.19 (7) 0.145	11.6±1.37 (6) 0.769	11.7±0.90 (8) 0.716	11.8±1.37 (7) 0.683
Lymphocytes (%) P Value	69.3±3.99 (6)	58.8±5.66 (8) 0.071	71.4±2.25 (7) 0.720	71.4±1.91 (8) 0.719	72.0±2.05 (7) 0.547
Platelets (10^3/mm3) P Value	11170±76.6 (6)	1100.88±55. 6 (8) 0.854	969.00±45.2 (7) 0.110	929.63±61.1 (8) 0.040	1168.29±81.3 (7) 0.659
Splenocyte proliferation (T/C ratio) P Value	2.92±0.747 (5)	0.76±0.0721 (5) 0.015	1.82±0.347 (4) 0.217	2.55±0.485 (6) 0.644	2.68±0.977 (4) 0.783
Bone marrow Cellularity (10^6/mm3) P Value	65.0±5.72 (4)	56.1±8.80 (4) 0.382	70.5 ±3.45 (4) 0.586	64.9±6.76 (5) 0.990	75.5±6.94 (5) 0.279

Table 4. Effect of high fat / high carbohydrate feeding on serum lipid profile and body composition – influence of flax seed oi

	CT	HF	HFF1	HFF2	HC
Triglycerides mg/dl P value	77.0±5.9 (5)	75.0±7.9 (7) 0.824	76.0±6.5 (6) 0.868	65.0±5.6 (7) 0.243	91.6±6.13 (7) 0.134
Cholesterol mg/dl P value	52.5±0.99 (6)	50.4±1.32 (8) 0.471	40.6±0.93 (8) 0.001	39.6±3.61 (7) 0.001	53.8±3.39 (8) 0.762
HDL / TC ratio P value	0.78±0.03 (6)	0.67±0.01 (8) 0.028	0.72±0.05 (7) 0.261	0.65±0.04 (6) 0.017	0.53±0.08 (7) 0.020
Fat% P value	12.7± 0.85 (6)	12.5±0.75 (7) 0.715	12.9±0 .40 (8) 0.392	11.7±0.49 (8) 0.378	14.7± 0.466 (7) 0.050
FFM P value	142.60±5.38 (6)	140.42±5.24 (7) 0.751	139.15±3.58 (8) 0.605	136.62±4.19 (8) 0.373	129.80±5.58 (7) 0.130
LBM P value	240.55±10.1 (6)	235.88±9.99 (7) 0.727	235.41±7.27 (8) 0.693	228.01±8.39 (8) 0.339	217.42±10.9 (7) 0.153

Table 5. Effect of high fat / high carbohydrate feeding on some plasma biochemical parameters-influence of flax seed oil

	CT	HF	HFF1	HFF2	HC
Urea mg/dl	28.40± 1.990 (5)	23.3± 1.66 (7)	21.0 ±2.809 (8)	18.86± 1.262 (7)	24.86± 2.340 (7)
P value		0.128	0.028	0.007	0.303
Creatinine mg/dl	0.64 ±0.081 (6)	0.70 ±0.042 (8)	0.60 ±0.072 (8)	0.64 ±0.088 (8)	0.77±0.086 (8)
P value		0.577	0.680	0.966	0.326
TP gm/dl	7.84±0.469 (6)	6.9 ±0.357 (4)	6.71 ±0.328 (7)	7.08±0.520 (7)	7.37± 0.119 (8)
P value		0.198	0.079	0.225	0.284
Alb gm/dl	4.17± 0.117 (6)	4.09 ±0.068 (8)	4.15 ±0.133 (7)	3.85± 0.580 (7)	4.45 ±0.128 (8)
P value		0.855	0.965	0.478	0.145
ALP IU/L	548±77 (6)	476± 59 (7)	492±56 (7)	641± 70 (6)	446± 48 (8)
P value		0.443	0.546	0.341	0.257
ALT IU/L	24.6 ±1.75 (5)	21.4±3.61 (7)	40.3 ±4.13 (7)	51.8±9.01 (8)	32.5±6.41 (6)
P value		0.738	0.107	0.007	0.305
AST IU/L	84 ±8.16 (6)	79.5± 5.12 (6)	90.8±8.78 (8)	104.30±15.0 (8)	94.1± 5.67 (8)
P value		0.788	0.667	0.203	0.313
TBIL mg/dl	0.32± 0.032 (6)	0.36± 0.069 (8)	0.41± 0.050 (8)	0.48±0.056 (8)	0.42 ±0.048 (8)
P value		0.572	0.245	0.065	0.105
Calcium mg/dl	10.4±0.26 (6)	9.95±0.33 (8)	9.76±0.31 (8)	8.13±0.50 (8)	8.99±0.740 (8)
P value		0.409	0.250	0.001	0.133

Table 6. Effect of feeding high fat / high carbohydrate diet on selective gut bacteria - influence of flax seed oil

Bacteria	CT	HF	HFF1	HFF2	HC
Lactobacillus $X10^8$ CFU	9.04±2.41 (5)	4.08±1.26 (6)	7.57±3.37 (6)	7.87±4.51 (6)	6.72±2.40 (5)
P Value		0.275	0.742	0..793	0.621
Bifidobacteria $X10^5$ CFU	95.60±30.12 (5)	98.17±37.42 (6)	108.83±40.84 (6)	157.00±50.63 (7)	100.00±51.56 (5)
P Value		0..969	0.839	0.334	0. 948
Enterobateriacea $X10^5$ CFU	0.00 (5)	18.74±5.40 (5)	10.50±5.77 (6)	3.00±1.76 (5)	1.60±0.60 (5)
P Value		0 .005	0 .091	0 725	0.916
Enterococcus $X10^6$ CFU	20.60±10.98 (5)	9.67±3.76 (6)	5.00±1.87 (5)	6.00±3.51 (7)	22.60±12.86 (5)
P Value		0.302	0.163	0.159	0.855
Streptococcus $X10^7$ CFU	0.98±0.32 (5)	0.70±0.30 (6)	0.30±0.15 (6)	0.49±0.18 (7)	1.16±0.48 (5)
P Value		0.509	0..117	0..233	0..684
Bacteroides (CT value)	20.20±0.56 (6)	18.59±0.93 (6)	18.64±0.85 (6)	18.48±0.59 (6)	18.91±0.58 (6)
P Value		0. 125	0.137	0.102	0. 217
Firmicutes (CT value)	16.01±0.53 (5)	14.89±0.25 (6)	14.56±0.24 (6)	14.13±0.18 (6)	16.34±0.67 (6)
P Value		0.074	0 .024	0.004	0.584

Tables 2 to 6: Values are mean ± SE. Figures in parentheses indicate sample size. CT = control ; HF= high fat diet HFF1 = high fat +flax seed oil 7.5ml/kg body weight; HFF2 = high fat +flax seed oil 15 ml/kg body weight; HC=high carbohydrate P Value = with respect to control group.

CONCLUSIONS

Effects of feeding high fat diet

In the rats fed a high fat diet (palmolein at 30% of diet by weight) there was a decrease in total WBC count, lymphocyte count, decreased splenocyte proliferation response to mitogen, increased neutrophil count in association with increase in gram negative bacteria, decrease in gram positive like lactobacilli.

The results of the present study show that both high palmolein and high starch diets have differential adverse effects on the host; while only high palmolein diet is found to impair immunity and increase inflammation, only high starch diet was found to effect body composition and lipid profile. The observed changes seen in HC diet given rats such as increased body fat %, increased triglycerides, decreased HDL are effects associated with an increased risk of cardiovascular diseases by a variety of mechanisms . From the results seen with palmolein we can conclude that a high palmolein diet has a more deleterious effect on the immune system in rats. Since probiotics like lactobacilli are known to positively influence immune system and that gram negative bacteria impair immune function, a correlation may be drawn between impairment in immune system and drop in the ceacal lactobacilli count and increase in gram negative enterobacteriaceae seen in the HF fed rats in the present study. The current study also goes to prove that independent of energy intakes both high fat or high carbohydrate intakes have adverse effects compared to a standard diet. The effects of dietary fats on the risk of coronary artery disease have traditionally been estimated from their effects on serum total cholesterol. However, their effects on gut bacteria which has implications in producing metabolic syndrome a relatively new concept was not known earlier. The current study also emphasizes the need to look into immunological as well as bacterial profile to assess the safety of dietary fats besides considering biochemical profile and body composition parameters. Also, for the first time we are showing the beneficial effects of flax seed oil on HF induced adverse effects which are probably mediated through gut bacteria.

From the results obtained in the present study, it can be envisaged that the adverse effects of feeding palmoil at 53 energy% in the diet (HF) are mediated through immune impairment and inflammatory response mediated through alteration in selective gut bacteria and that flax oil has a prophylactic role and may serve as an alternative dietary strategy in the management of diseases caused by high fat induced bacterial changes probably by serving as a prebiotic. Incorporation of flax oil into the preparation of high fat diets and also use of symbiotic formulations with flax oil may also have potential clinical utility in normalizing many of the high fat induced adverse effects.

V BASIC STUDIES

1 ENHANCING DIETARY IRON AND ZINC BIOAVAILABILITY IN INDIAN CHILDREN

Widespread iron deficiency anemia is attributed to habitual consumption of diets rich in inhibitors of non-heme iron absorption. Iron and zinc deficiencies usually occur concurrently in the Indian population because the dietary factors that impair iron absorption often will also adversely affect zinc absorption. This has significant impact on adolescents due to the higher requirement of these minerals during their period of rapid growth. Furthermore, the bioavailability of iron and zinc from diets vary according to age and gender leading to uncertainty about optimal nutrient requirement. A limited number of studies have been carried out in vulnerable segments of the population, especially during adolescence to assess the extent of iron and zinc bioavailability.

AIMS AND OBJECTIVES

Primary Objectives

a) To measure the bioavailability of iron and zinc from a representative Indian diet in 13 - 15 years old children using stable isotopes of iron and zinc.

b) To measure the change in bioavailability of iron and zinc achievable by feasible dietary diversification of a representative Indian diet using stable isotopes of iron and zinc.

Secondary Objectives

a) To evaluate the relationships among iron status, zinc status and iron and zinc absorption in boys and girls.

b) To determine the tolerability of feasible dietary modifications by children using a 5-point hedonic scale questionnaire.

METHODOLOGY

Sample size

A sample size of 14 participants for each sex for paired comparison was arrived at by assuming that the intervention would lead to a doubling of iron absorption from 3 to 6%, with a mean difference of 3% and an SD of 3%, and with an a error of 0.05 and power of 90%. The final sample size of 16 for enrollment was calculated considering a possible dropout rate of 20% during the study.

Study Design

The study was carried out in two single-gender residential schools among the students of grades 7-9 (13-15y) under the Andhra Pradesh Social Welfare Residential Institution Society during the academic years April 2010 to March 2012. Ethical approvals were obtained from the Institutional Review Board of NIN and CNRC, Baylor College of Medicine, Houston, US. Approvals from the Secretary, Andhra Pradesh Social Welfare Residential Educational Institutional Society and the principals of the two selected schools were also obtained. Written consents from the participants and assents from their parents were obtained. A total of 102 girls and 72 boys aged 13-15 y consented for the study and were dewormed. Eligible 42 girls and 21 boys were enrolled, from which 16 girls and 16 boys were randomly allocated for the study (Figure 1).

Regular and Modified meals

Participants were fed a standardized rice meal (regular), and the same meal with 100 g of guava fruit (modified) for assessing iron and zinc absorption in a 36-day trial period.

Dosing and Sample Collection

On day 1 of the study, 10 mL of fasting (12 h) blood was collected at 7 am followed by i.v. administration of ^{70}Zn (400 mg) to all participants. During the lunch meal, 2 mL of ^{67}Zn (1 mg) and 1.7 mL of ^{57}Fe (3.4 mg) were given orally, in tandem, in glucose water (100 g/L) along with the pre-weighed lunch. Dosing of iron (^{57}Fe) was repeated the next day with an identical freshly cooked lunch meal. On day 3 at 7 am, after 12 h of fasting, a reference dose of 0.8 mL of ^{58}Fe (1.6 mg), mixed in 100 mL of commercial fruit juice (ascorbic acid, 100 mg/100 mL), was administered orally. Participants were allowed to have breakfast 2 h after the dosing. Provision of modified meal was initiated on day 18 by using the above protocol, except that the reference dose was not administered on day 20. Urine samples (50 mL) were collected between 10 and 11am on days 3–6 and on days 20–23. Ten milliliters of fasting blood was collected into standard heparinised vials by using mineral-free syringes on day 18 and also day 36 (Figure 1).

Laboratory Analyses

Nutrient analysis in the composite diets was carried out in lyophilized samples, except for ascorbic acid which was estimated in freshly homogenized samples. Ascorbic acid content was estimated by spectro photometric method, total protein by the Kjeldal and iron and zinc by atomic absorption spectrometry. Phytate was estimated as phosphorus by anion exchange followed by acid digestion. Hemoglobin concentration was estimated in whole blood while concentrations of ferritin, soluble transferrin receptor (sTfR), hepcidin, C-reactive protein (CRP), vitamin B12, folate, vitamin C and A were estimated in plasma at NIN. Bioavailability of iron in RBC (n=96) and zinc in urine samples (n=256) were assessed at the collaborating laboratory BCM, Houston, Texas, US. The relative abundance of the stable isotopes of iron in blood and zinc in urine were measured using thermal ionization mass spectrometry (TIMS) and coupled plasma mass spectrometry (ICP-MS), respectively.

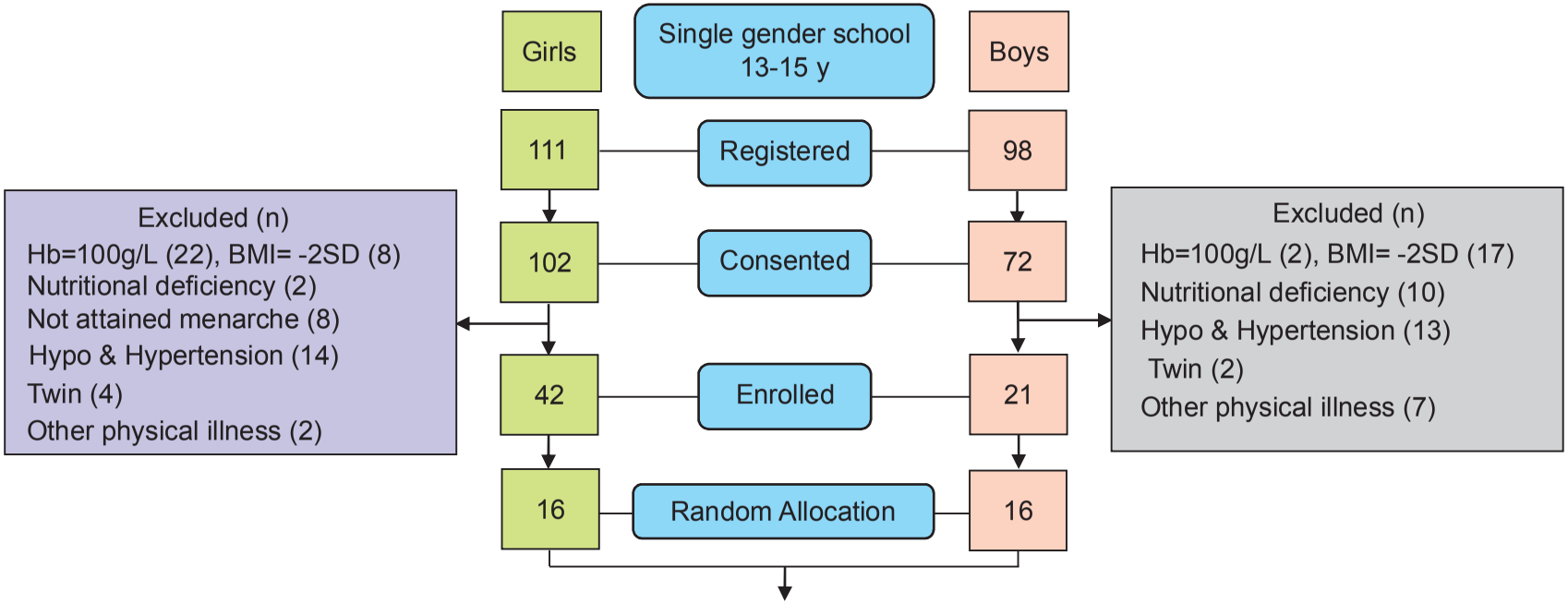
Evaluation of tolerability of dietary modification

Ten different chutneys, 7 vegetable dhals and 7 types of salads were prepared using minimally processed ingredients. Ascorbic acid concentration was estimated. These preparations were then subjected to sensory evaluation on a 7-point Hedonic scale by an institutional panel. Based on the panel's score and ascorbic acid content of >20mg/100g, 5 types in each preparation of chutney, dhals and salads were selected. Five fruits of high vitamin-C content were also listed. They were tested for preference using a self administered questionnaire among 150 girls of 13-15 y. They were asked to rank all the selected preparations according to their likings. From the highly preferred dishes, 2 types of minimally processed chutneys, 1 type of vegetable dhal, 1 type of salad and 1 fruit along with regularly served recipe were subjected to 5-days tolerability testing among same participants using self administered questionnaire.

STATISTICAL ANALYSES

Data was analyzed using SPSS for Windows version 19.0 (Chicago Inc. USA). Student's t-test was used for comparing means. Paired t-test was used to test the differences in absorption between the regular and modified meals and two- way repeated measures ANOVA to determine the effects of diet (regular vs modified) and gender (female vs male) and their interaction on the absorption of iron and zinc. To test the relation between biomarkers and absorption multiple linear regression modelling with adjustment for confounding variables was used. Pooled data of biomarkers and absorption was tested by Pearson correlation coefficient. The relation between phytate and mineral absorption was tested using Spearman's rank correlation.

Figure 1. Study design, time points of dosing and sample collection



Time / Day	Regular Meal				Modified Meal		Regular Meal	
	1	2	3	4 – 5	18	19	20 – 22	36
At 7h on12 hours fasting	Blood / ^{70}Zn (IV)	–	^{58}Fe (oral)	–	Blood / ^{70}Zn (IV)	–	–	Blood
At 10 - 11 h	–	–	Urine	Urine	–	–	Urine	–
During meal at 13 h	^{67}Zn and ^{57}Fe in tandem (oral)	^{57}Fe (oral)	–	–	^{67}Zn and ^{57}Fe in tandem (oral)	^{57}Fe (oral)	–	–

RESULTS

Nutrient composition of meals

Iron and zinc contents of the regular and modified lunch were similar. Protein and phytate contents of the meals were also similar. The modified meal had about 20 fold higher ascorbic acid content compared to the regular meal. The molar ratio of iron: phytic acid was greater than 1:1 in both the meals and the modified meal had 20 times greater ascorbic acid content than the regular meal (Table 1).

Table 1. Meal and nutrient composition of regular and modified meals

Details	Girls		Boys	
	Regular	Modified	Regular	Modified
Meal composition				
Rice, [1] g	450	450	400	400
Vegetable Curry, [2] g	100	100	100	100
Sambar, [3] g	100	100	150	150
Butter milk/ Yogurt, [4] g	100	100	200	200
Guava, g	–	100	–	100
Nutrient Composition				
Protein, g / meal	10.2	9.6	9.8	9.8
Iron, mg / meal	10.8	13.3	11.4	10.0
Zinc, mg / meal	2.6	2.7	2.8	2.8
Ascorbic acid, mg / meal	7.8	188.0*	10.4	190.4*
Phytate, g / meal	0.3	0.2	0.4	0.3
Fe : PA : AA [5]	1 : 2.4 : 0.2	1 : 1.3 : 4.6	1 : 3 : 0.3	1 : 2.5 : 6

[1]Rice - equivalent to dry weight, 130g for boys and 150g for girls.

[2]Vegetable curry - 80g potato and 20 g tomato.

[3]*Sambar* - a broth prepared by combining 30g dehusked split pigeon peas, , tamarind pulp and 15g of bottle gourd. The other ingredients used in the preparation are onions, green chillies, red chilies, turmeric, iodized salt, spice powder and peanut oil to taste.

[4]Buttermilk was served to boys by constituting yogurt in water as 1:1.

[5]Fe : PA : AA – non- heme iron : phytic acid : ascorbic acid.

*Significantly different from regular diet $P<0.05$ by 't' test.

Subject characteristics, iron and zinc status

The mean BMI of girls was significantly higher ($P<0.05$) compared to boys. All the subjects had weight for age Z- scores between > -2SD and <+1SD, except one girl with a score >+1SD. Body weights were lower than the Indian reference body weights for adolescents. However, the BMI in girls was similar and for boys it was lower than the Indian reference value (Table 2). No significant differences were found in iron status and plasma zinc between genders. Among boys, 3 were anemic, (Hb <120 g/L), 5 had low ferritins (<12 µg/L) and 14 had elevated sTfR (29.5 nmol/L). Among the girls, 2 had anemia 7 had low ferritins and 13 had elevated sTfR. Plasma hepcidin concentration was 2.5 times higher ($P<0.05$) and ascorbic acid was significantly lower ($P<0.05$) among boys, compared to girls. The mean CRP levels were comparable between genders and none of the participants had CRP levels > 10 mg/L. There were no gender differences in serum folate, vitamin B-12 and retinol concentrations (Table 3).

Table 2. Anthropometric characteristics of the participants and their comparison with Indian and FAO/ WHO reference values[1]

Characteristics	Girls			Boys		
	Present Study	Reference Value		Present Study	Reference Value	
		Indian[2]	FAO/ WHO[3]		Indian [2]	FAO/WHO[3]
Age, y	14.2 - 0.82	13-15	11-14	13.9 - 0.75	13-15	11-14
Height, cm	149 - 4.9	156.3	–	157 - 9.3	162.1	–
Weight, kg	43.3 - 4.15	46.6	46.1	40.1 - 4.85	47.6	45
BMI [4], kg / m²	19.5 - 1.91	19.0 16.8 - 2.3[5]	–	16.7 - 0.97*	18.1 15.7 - 2.1[5]	–

[1]Values are mean ±SD, n=16
[2]95th percentile value based on national reference data (9).
[3] FAO/WHO mean value (26).
[4]BMI for age calculated based on WHO Z-score scale.
[5]Rural India (27)
All boys were normal (-2Z to +1Z) and only one girl showed > +1Z score suggestive of overweight.
*Different from girls, $P<0.05$ by t test.

Table 3. Biomarkers of iron status, plasma hepcidin, zinc, CRP, ascorbic acid, folate, vitamin B_{12} and retinol of the girls and boys at baseline[1]

Biomarkers	Girls	Boys
Hemoglobin[2], g/L	129 - 7.8	126 - 7.1
Ferritin[3], µg/L	12.3 (9.3, 17.2)	16.3 (9.3, 22.3)
sTfR[3], nmol/L	37.1 (30.3, 50.7)	37.7 (33.4, 45.1)
Hepcidin[3], µg/L	7.5 (3.7, 11.0)	19.9 (10.3, 33.1) *
Zinc[2], µmol/L	13.7 - 1.0	14.0 - 2.2
CRP[3], mg/L	0.40 (0.40, 0.75)	0.79 (0.40, 2.96)
Ascorbic acid[3], µmol/L	30.4 (21.6, 36.1)	17.0 (12.2, 20.5) *
Folate[3], nmol/L	11.3 (10.0, 13.1)	12.0 (10.9, 12.9)
Vitamin B-12[3], pmol/L	127 (107, 150)	140 (112, 183)
Retinol[2], µmol/L	1.0 - 0.27	0.9 - 0.24

[1]Values are mean ± SD or median (IQR), n=16.
[2]Normally distributed variables 't'test was applied.
[3]Skewed variables non-parametric Man-Whitney U test was applied.
*Different from girls, $P<0.05$.

Iron and zinc absorption

The modified meal enhanced iron bioavailability significantly compared to a regular meal in girls and in boys ($P<0.05$). Fractional zinc absorption did not differ between regular and modified meal in either sex (Table 4).There was no gender difference in iron bioavailability. Fractional absorption of zinc tended to be greater in males than females ($P = 0.054$).

Iron and zinc status and absorption

Pooled data of iron status biomarkers did not show any relation with iron bioavailability, except for a positive correlation (r= 0.56, $P=0.001$) between sTfR concentration and iron bioavailability from the reference dose. However, in girls, log plasma ferritin and iron bioavailability showed negative correlation when a modified meal was consumed (r= - 0.61, $P = 0.016$). These results

Table 4. Effect of modification of rice meal with guava on percent iron bioavailability and factional zinc absorption in adolescents

Mineral absorption	Girls (n=15)		Boys (n=14)		Two-way Repeated Measure ANOVA P Values		
	Regular	Modified	Regular	Modified	Diet	Gender	Diet X Gender
Iron	9.7 - 6.5	23.9- 11.2	8.6 - 4.1	19.2 - 8.4	0.0001	0.125	0.459
Difference*		14.2- 14.5		10.6 - 10.4			
Zinc	26.9 - 6.4	27.3- 7.4	32.5 - 16.6	37.1 - 3.0	0.122	0.054	0.208
Difference#		0.5 - 6.3		4.6 - 10.9			
Reference dose ^{58}Fe#	58.2 - 22.2		48.9 - 16.0				

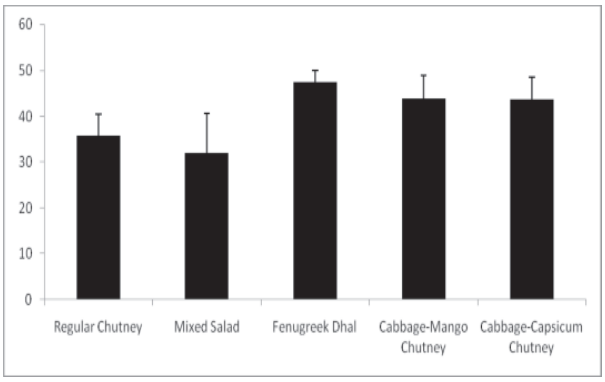
Values are presented as mean ±SD, * by paired t test *P*=0.002. The effect of diet, gender and their interactions was tested by two-way repeated measure ANOVA, # not significant

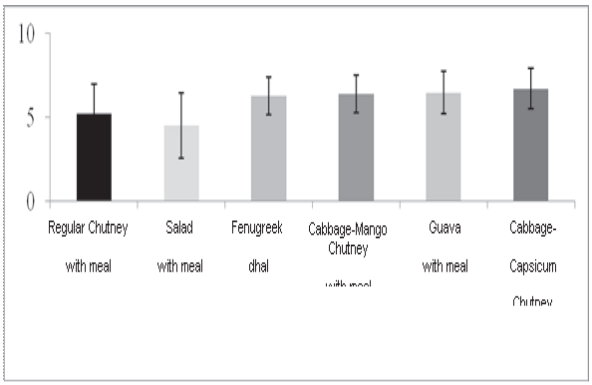
suggest that iron status can influence its absorption, though the effects are not very obvious, and that these relationships may exist only when dietary iron is bio-available, which presents iron in a more accessible form for absorption at enterocyte. Another iron regulatory factor, hepcidin showed a significant correlation with iron absorption (standardized beta = - 0.63, *P*=0.001, R^2 = 0.40) after adjusting for hemoglobin and log values of ferritin, sTfR, ascorbic acid, vitamin B-12, folate and CRP as confounders [unstandardized beta = - 31.4 (95% CI = - 49, -13.9)]. There was no significant association between fractional zinc absorption and plasma zinc concentration in either sex. There was no significant relation between intakes of phytate and iron and zinc absorption.

Acceptability / Tolerability of feasible dietary modification

The chutneys prepared with cabbage-mango had 66.9 mg/100 g of vitamin C and that with cabbage-capsicum 52.9 mg/100g. The participants scored a mean ± SD of 43.8 ± 5.01 and 43.7 ± 4.8 respectively for cabbage-mango chutney and cabbage-capsicum chutney as against a score of 35.8 ± 4.53 for the regular chutney served to them (Figure-2).

Figure 2. Acceptability of meal modified by minimally processed preparations and comparison with that of regular meal





CONCLUSIONS

Dietary diversification of a habitual rice-based meal with 100 g of guava enhanced bioavailability of non-heme iron but not zinc. There was no gender difference in the absorption of iron and zinc. Hepcidin concentration significantly predicted iron absorption. The participants well tolerated inclusion of minimally processed vegetables high in ascorbic acid in every meal. Long-term studies are needed to assess the impact of daily consumption of fruits rich in ascorbic acid along with nutrition education to translate these findings into a national strategy to control iron deficiency anemia.

2 ISOLATION AND CHARACTERIZATION OF AN IRON ABSORPTION ENHANCER FROM HUMAN MILK

The poor solubility of ferric iron at physiological pH coupled with dietary chelators such as phytic acid and polyphenols limits the absorption of iron. Further, reduction of ferric iron is also necessary for the uptake at the enterocyte through divalent metal ion transporter 1 (DMT1). In tune with these observations the breast milk iron reported to be distributed predominantly in low molecular weight fractions, could be bound to peptides, amino acids or other components. Recently, the ferric reductase activity in low molecular breast milk fractions and provided the evidence for its association with enhanced ferric iron solubility and uptake in caco-2 cells were demonstrated. It's known that peptides (such as casein phospho-peptides) generated during gastric/intestinal digestion of milk proteins binds and enhances the absorption of iron. However, the fact that the ferric reductase or ferric iron solubilization activities in undigested human milk fractions implies the presence of specific native milk factor or factors that are primarily responsible in enhancing the ferric iron uptake was demonstrated. However, the mechanism by which factor reduces and thus enhances the iron absorption remains to be studied.

In order to get further insight in to the nature of iron binding component and mechanism of ferric iron reduction in the presence of low molecular weight milk fraction induced iron uptake, the effect of temperature, extracellular ferrous iron chelator and Dcytb neutralization (a duodenal ferric reductase, essential for iron absorption) on iron uptake was studied.

OBJECTIVES

1. Purification and primary structure elucidation of ferric reductase from human milk
2. To understand the enzymatic characteristics and mechanism of human milk ferric reductase induced iron absorption
3. To demonstrate the ferric reductase and iron absorption enhancing activities with *in vitro* synthesized compound in comparison to that isolated from human milk

METHODS

***Human milk fractionation*:** Aliquots of surplus human milk were collected after taking the consent of all the donors, and pooled. Collection of human milk samples in this manner was approved by the Committee on Use of Human Subjects in Research of Gandhi Hospital, Secunderabad. Aliquots of pooled human milk (50 mL) were fractionated by ultra filtration through 5kDa cutoff filters as described previously. The protein concentration in various milk fractions was assessed by micro BCA kit method (Sigma Chemical Co.) using BSA as a standard.

Heat denaturation: The 5kD cutoff membrane filtrate (5kF) was boiled in water bath for 20 min and immediately cooled on ice.

***Ferric reductase assay*:** Ferric iron reduction was measured as described previously. Briefly, the reaction mixture contained 50 µmole/L ferric iron (ferric chloride), ferrozine (500 µmole/L) in 50 mmole/L MES pH 6.5 in the presence and absence of 50 µL of milk fractions in the absence or presence of 0-100 µmole/L $ZnSO_4$. The reaction was carried out at 25 °C for 10 min in a micro plate based assay, while continuously monitoring absorbance at 562 nm using micro-plate reader (BioTek, Model# Powerwave HT-1). The percent (%) inhibition of ferric iron reduction was calculated by assuming the total activity in 5kF fractions as 100%.

Iron solubilization: Radio labeled ferric iron solubilization was measured as described previously. Briefly, 25 µL aliquots of the 5kF (either native or heat denatured) were diluted with 50 mmole/L MES buffer pH 6.5, supplemented with 25 µmole/L (traced with 50 nCi ^{59}FeCl3) in the absence and presence of 0-100 µmole/L zinc, and incubated for 30 min at 37°C. At the end of incubation the samples centrifuged at 15,000*g* at 4°C for 15 min and the supernatant solution (800 µL) was mixed with 5 mL of Brays mixture and counted in liquid scintillation counter (Perkin Elmer Model# TRICARB 2900TR). The percent (%) solubilization of iron was calculated by assuming the solubility of iron in the presence of 5kF fraction as 100%.

Caco-2 cell iron uptake: Caco-2 cells were grown in 6-well culture plates and used for the experiments between 13-14 days post-confluence as described previously. Ferric chloride (2.5 mmole/L stock in 10 mmole/L HCl traced with 50 nCi 59FeCl3) was diluted to a final concentration of 100 µmole/L with MES buffer (50 mmole/L, pH 6.5) in the absence (blank) or presence of 50 µL of 5kF and/or zinc (50 µmole/L) and then fed to the differentiated Caco-2 cells for a period of 2h. After the incubation the monolayer's were washed with ice-cold phosphate buffer saline containing 10 mmole/L bathophenonthroline (to remove non-specifically bound iron), harvested by scraping and 59Fe radioactivity counted in liquid scintillation counter. The % uptake was calculated assuming the uptake in control cells as 100%.

DcytB neutralization: To test the role of DcytB on low molecular weight human milk fraction induced iron uptake, the iron uptake studies were carried in the presence and absence of DcytB blocking antibody as described previously (Turi et al., 2006). Differentiated Caco-2 cells grown on 12-well plates were treated with a DcytB blocking antibody (1:100 dilution) in minimum essential medium (MEM) for 30 min, and then iron uptake was studied as described above in the absence and presence of either native or boiled 5kF fractions. The % uptake was calculated assuming the uptake in control cells as 100%.

Citrate lyase/oxaloacetate decarboxylase treatment of 5kF fraction: Citrate lyase (Klebsiella neumoniae, E.C. No. 232-740-7, Sigma#C0897)/ oxaloacetate decarboxylaase (Pseudomonas sp. E.C. No. 4-1-1-3, Sigma#O4878) digestion was carried out as described previously with modifications[23]. The 5kF fraction was diluted with 1:1 (v/v) with 20 mM HEPES buffer pH 7.8, followed by addition of 2 units each of citrate lyase and oxaloacetate decarboxylase (pre-dissolved in 10 mmol/L HEPES buffer pH 7.8). The samples were incubated for 28 h at room temperature. At the end of incubation, the ferric iron reduction, solubility and Caco-2 cell iron uptake were assessed as described above with citrate lyase digested 5kF fraction and 100 µmol/L of standard citric acid.

Estimation of citric acid: The citric acid content in 5kF fractions before and after treatment with citrate lyase/oxaloacetate decarboxylase treatment was estimated by pyridine-acetic anhydride method.

STATISTICS

All the experiments were performed in triplicates and repeated at least once to generate 6 observations. Mean and SD were calculated using Microsoft Excel and the data was analyzed using One-way ANOVA followed by least significant differences (LSD) test, using SPSS package (Version 2007). The results were considered significant if the $p<0.05$.

RESULTS

Isolation and characterization of low molecular weight (LMW) human milk fractions: The protein content in 5kF fraction (filtrate obtained by ultrafiltration of human milk whey through 5kDa cutoff membrane) was 1.12 mg/mL while it was 1.9 mg/mL in 10kF fraction. The specific activity of ferric iron reduction and solubilization was higher with 5kF (89%/mg protein) fraction compared to

10kF fraction (53%/mg protein). Therefore, 5kF fraction was used for all the subsequent studies. The 5kF human milk whey fraction contained 42% iron (9.24±2.1 µg/100 mL) and 17% zinc (40.1± 4.3 µg/100 mL) present in the whole human milk (28±11 µg/100 mL iron and 241±18 µg/100 mL zinc, respectively).

Effect of heat denaturation and zinc on LMW human milk fraction induced ferric iron reduction and solubilization: 5kF fractions increased the reduction (100%±5.2) and solubilization (100%±2.1) of ferric iron compared to control, $FeCl_3$ (1.8% and 4.2%, respectively, Fig. 1). Heat denaturation inhibited the 5kF induced ferric iron reduction but not iron solubilization activity. In contrast, addition of 5-100 µmol/L zinc dose dependently inhibited the 5kF induced ferric iron reduction and solubilization (Fig. 1). Similarly, zinc also inhibited the ferric iron solubilization induced by heat denatured 5kF fractions (data not shown).

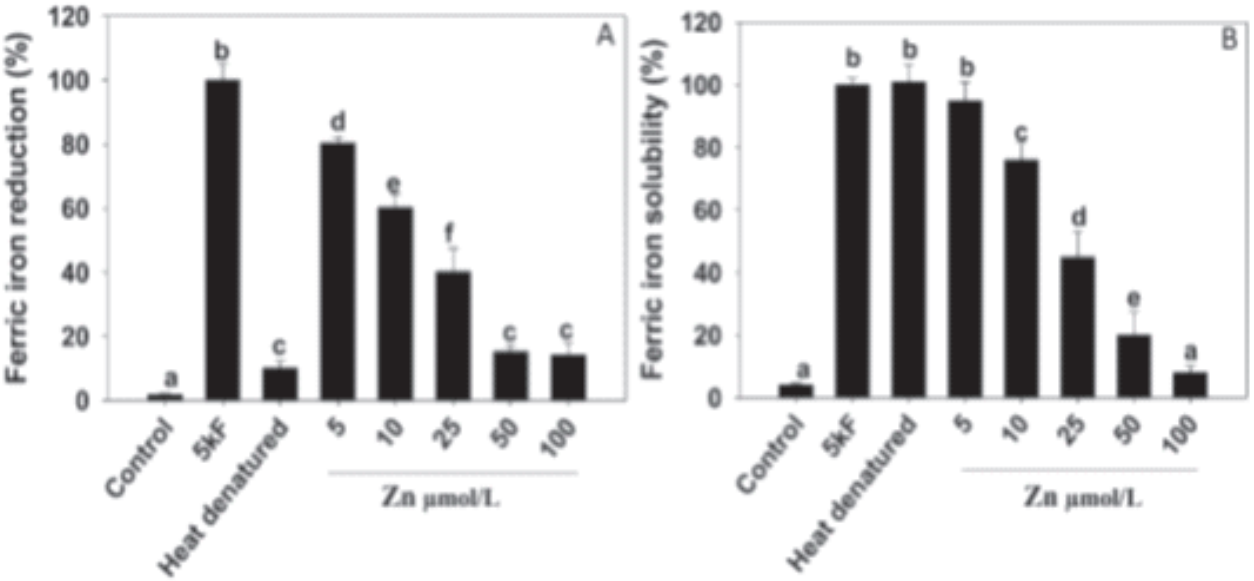


Figure 1: Effect of heat denaturation and zinc on low molecular weight human milk fraction induced ferric iron reduction and solubility: Ferric iron reduction (A) and solubility (B) were assayed in native and heat denatured 5kF fractions in the presence or absence of 0-100 µmol/L Zn as described in methods. The % reduction and solubility were computed by considering the activity in native 5kF fractions as 100%. The bars indicate mean+SD and bars with different superscripts are significantly different ($P < 0.05$).

Effect of heat denaturation, zinc and DcytB neutralizing antibody on LMW human milk fraction induced ferric iron uptake in Caco-2 cells: Native (270±21%) and heat denatured 5kF fraction (254%±28) increased the ferric iron uptake to the same extent in Caco-2 cells compared to control (100%±10.5), as assessed by ^{59}Fe uptake (Fig. 2A). Addition of 100 µmol/L zinc (1:1 ratio of iron to zinc) significantly inhibited the ^{59}Fe uptake in Caco-2 cells from control, native and heat denatured 5kF fractions (Fig. 2A). Similarly, zinc also inhibited the iron uptake from heat denatured 5kF fraction (data now shown). Pre-treatment of Caco-2 cells with DcytB neutralizing antibody reduced the ^{59}Fe iron uptake in control (42%±4.4), native (212%±12.6) and heat denatured 5kF fraction (137%±9.6) compared to respective controls with out antibody (Fig. 2B). However, the extent of inhibition in ^{59}Fe iron uptake due to DcytB neutralization was lower in native 5kF fraction (21%) compared to heat denatured 5kF fraction (44%).

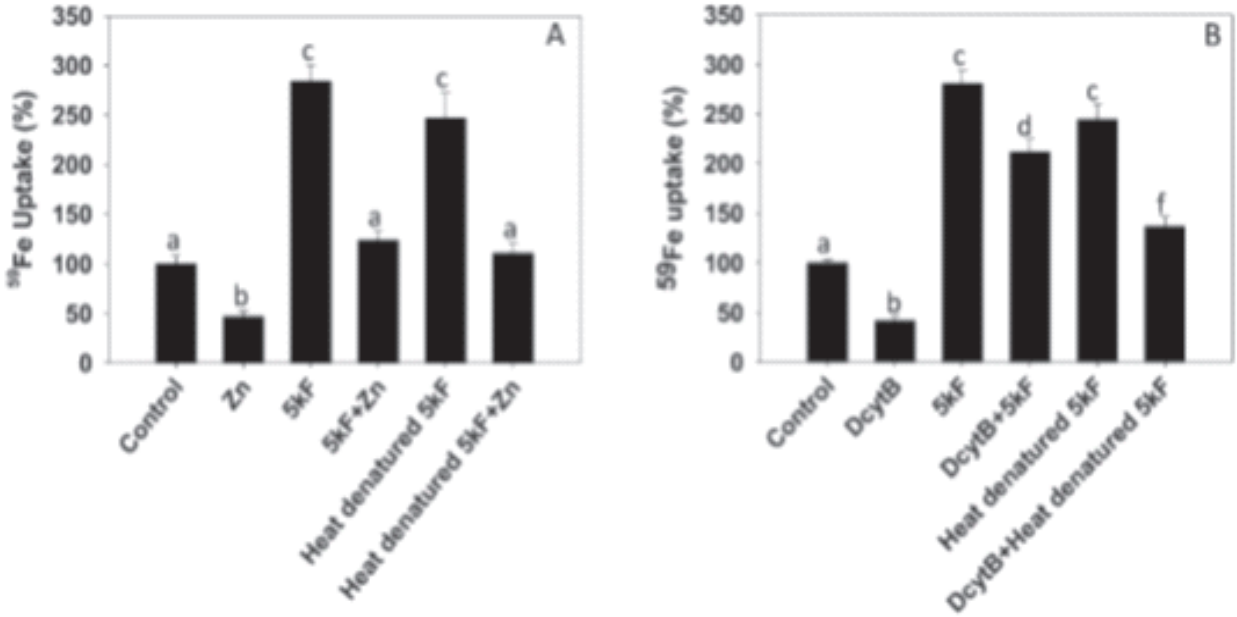


Figure 2: Effect of heat denaturation and zinc on LMW human milk fraction induced iron uptake in Caco-2 cells: (A). Differentiated Caco-2 cells were incubated with 100 mol/L $FeCl_3$ in the absence (control) or presence of 100 µL of 5kF milk fractions and/or zinc (100 mol/L) for 2h. (B). ^{59}Fe uptake studies were done in differentiated Caco-2 cells either in presence or absence of DcytB antibodies. At the end of incubation, the cell associated ^{59}Fe radioactivity was counted as described in methods. The percent uptake was calculated by assuming the uptake in control (only $FeCl_3$) as 100%. The bars indicate mean+SD of 6 observations and the bars with different superscripts differ significantly ($p<0.05$).

Effect of citrate lyase/oxaloacetate decarboxylase digestion of LMW human milk fraction and citric acid on ferric iron reduction, solubilization and uptake in Caco-2 cells: Citric acid content of 5kF fraction was 242 µg/mL, but was reduced to undetectable levels after enzymatic digestion. Citrate lyase/oxaloacetate decarboxylase treatment significantly inhibited the 5kF fraction induced ferric iron reduction, solubilization and ^{59}Fe iron uptake in Caco-2 cells (Fig. 3). On the other hand standard citric acid (1:1 molar ratio of iron to citric acid) significantly increased the iron solubility and uptake in Caco-2 cells without reducing the ferric iron (Fig. 3).

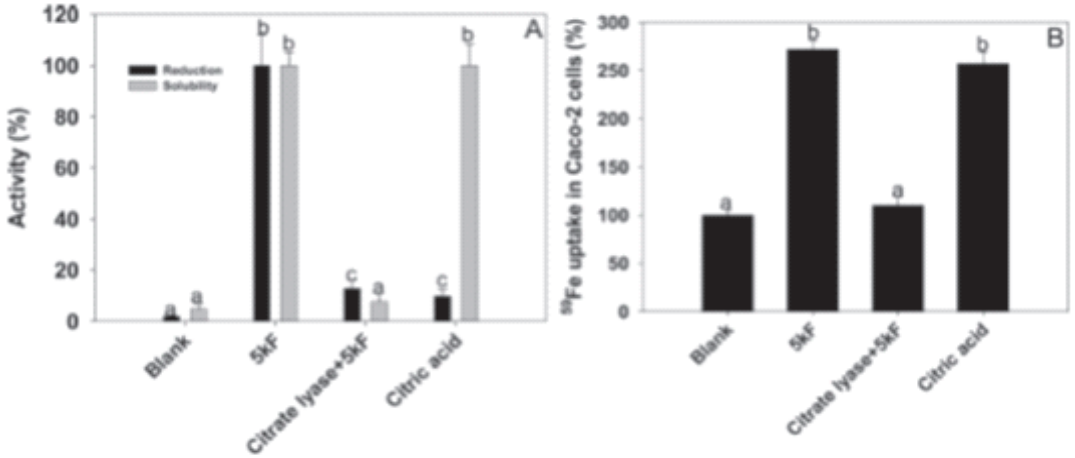


Figure 3: Effect of citrate lyase/oxaloacetate decarboxylase digestion on low molecular weight human milk fraction induced ferric iron reduction, solubilization and uptake in Caco-2 cells. The 5kF milk fraction were diluted to 1:1 (v/v) with 20 mmol/L HEPES buffer pH 7.8 followed

by addition of 2 units each of citrate lyase and oxaloacetate decarboxylase reconstituted in 10 mmol/L HEPES pH 7.8. The solutions were incubated at room temperature for a period of 28 h. At the end of incubation, ferric iron reduction, solubilization (A) and Caco-2 cell uptake (B) were measured as described in methods. The percent reduction and solubility was computed by assuming the activity in untreated 5kF fraction as 100%. The % uptake of ferric iron was computed by assuming the uptake in control (only $FeCl_3$) as 100%. The bars indicate mean+SD of 6 observations and the bars with different superscripts differ significantly ($p<0.05$).

CONCLUSION

Together, these results demonstrate that citric acid present in the LMW human milk fraction solubilizes the ferric iron at neutral pH, while other heat labile components present in human milk reduce the ferric iron (complementary to intestinal DcytB) leading to enhanced intestinal cell uptake. Further, these studies also highlight the role of DcytB during iron absorption from soluble ferric iron chelates.

3 STRESS, ALLOSTATIC LOAD AND MICRONUTRIENT STATUS; IMPACT OF DIETARY ADVICE

Due to the long lasting effects, adolescent stress is gaining importance in the area of stress biology. The index allostatic load postulated to quantify the cumulative physiological burden/ deregulation (AL) proves to be beneficial in the context. Psychological stress is a major component of AL and the index has demonstrated greater prediction of morbidity and mortality in the elderly and is currently used in other age groups as well. Adolescent age group is considered as susceptible to micronutrient deficiencies in India (NNMB, 2003; NNMB, 2006). Therefore, there was a need to understand how the greater demand due to physical growth and a consequent low status of micronutrients influences stress in adolescents and the changes that can be caused due to a change in micronutrient status.

HYPOTHESIS

High psychological stress will lead to allostatic load among higher secondary students of 15-19y age groups and specific advice to improve knowledge will improve micronutrient status and reduce high stress and allostatic load.

OBJECTIVES

1. To elucidate the relationship between psychological stress and i) allostatic load ii) micronutrient status.
2. To impart knowledge on micronutrients and to assess the impact on biomarkers of micronutrient status, allostatic load and stress parameters.

The study had two secondary objectives i.e.

i) To identify the target group for stress study among higher secondary students – pilot study and

ii) Construction and validation of a knowledge questionnaire on micronutrients.

Since, there were no studies to arrive at appropriate sample size, a pilot study was carried out, the results of which had been presented in Annual report 2010. The sample size of 353 was calculated for the main study based on this pilot study. To facilitate objective 2, a nutrition knowledge questionnaire was developed using the principles of psychometry which was found to have a positive association with plasma retinol status. The results were presented in Annual report 2011.

The one year prospective institution based study was carried out in 5 Government-run schools of Greater Hyderabad Municipal Corporation (GHMC). Clinical examination was carried out to apply inclusion/ exclusion criteria. The sampling used was a probability-proportional to size of school recruiting 370 adolescent boys (15-19y). A sub-sample (N=145) provided blood samples (10mL) and 12 h urine samples (N=107). The general characteristics of the population with respect to anthropometry, micronutrient status and changes after one year had been presented in Annual report 2012. The present report will concentrate on the three-way relationship between stress, allostatic load and micronutrient status and the impact of nutrition education on these three variables.

In order to elucidate the association between stress and allostatic load, the behavioural parameters studied were perceived stress measured by PSS-14, psychological morbidity by GHQ-12, stressful life events by ALESS and coping strategies by CSS scale. All scales were pre-tested before use. The allostatic load variables studied were DHEAS, IL-6, CRP, leptin, total cholesterol, HDL cholesterol, blood pressure and BMI. 12-hr urine samples were analyzed for cortisol. The composite index of allostatic load was created using the high risk quartile method, scoring the number of variables for which the participant falls into the highest quartile. Categorical scores of allostatic load ($\leq$2, >3) and mean values of stress variables were analyzed.

Association between micronutrient status, stress and allostatic load

The relationship between psychological stress and micronutrient status was elucidated using regression models and path analysis (AMOS, SPSS Inc. Chicago, USA). A positive association was observed between approach coping and vitamin B-12 status, independent of age, SLI, nutritional status and anaemia. Even though reports relating micronutrients such as vitamin B-12 to mental health and cognitive functioning and depression are plenty among elderly, coping has not been studied so-far in relation to micronutrient status. Stress may not be under the control of the individual, but coping processes are. Therefore, the role of vitamin B-12 needs to be probed in depth.

Regression analysis and path analysis elucidated a positive association between stress and inflammatory markers as well as hepcidin (Figure 1). However this pathway did not extend up to hypoferraemia as has been postulated for anaemia of inflammation. It is speculated that this lack of association could be due to the over-riding effects of iron deficiency as evidenced by hypoferritinemia of 36 % in this study population. Ferritin has been previously shown to have a critical role in iron absorption.

Impact of nutrition education intervention on biomarkers of micronutrient status and changes in allostatic load and stress parameters after 1 year

To understand whether a change in micronutrient status will bring about a change in stress and allostatic load, a multi-component nutrition education intervention utilizing a quasi-experimental design was carried out to enhance their knowledge on micronutrients was provided to the students

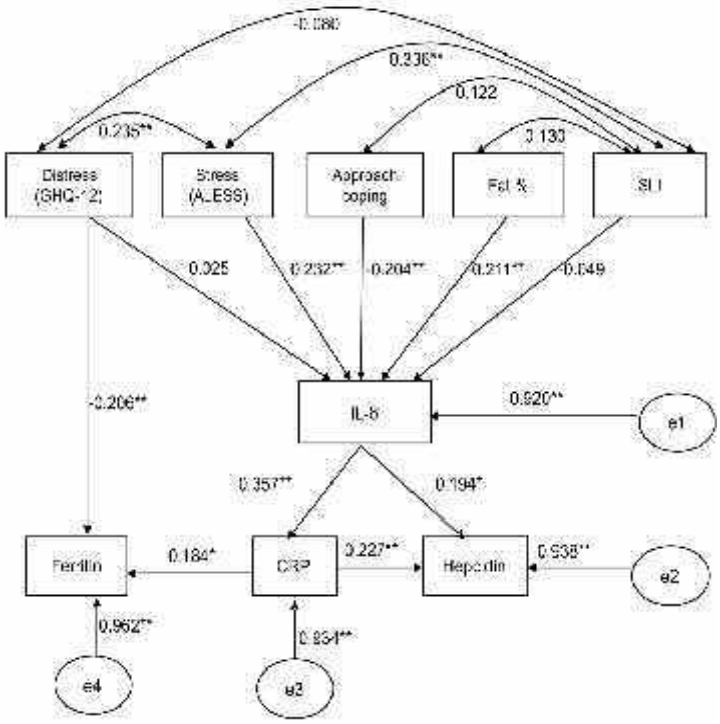
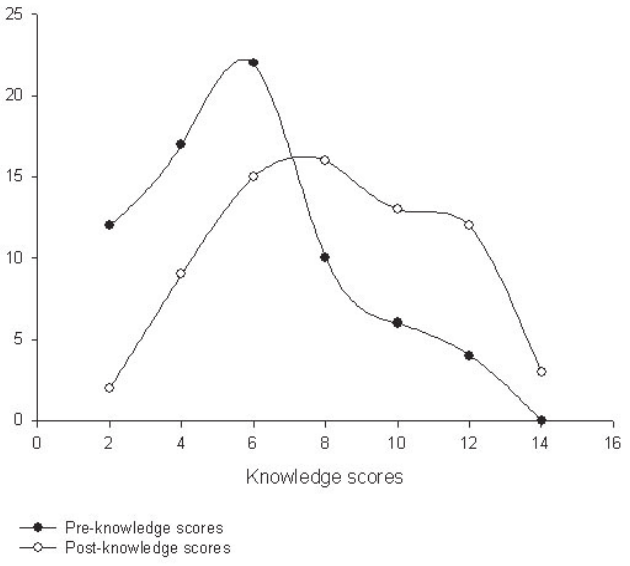


Figure 1. Path diagram showing the link between ALESS and hepcidin through IL-6. Measured variables are given in rectangular box, residual error 'e' in ovals. The single-headed arrows represent the dependencies between variables. Variables IL-6, hepcidin, CRP, ferritin and SLI were log transformed before analysis. The model showed a moderate fit (RMSEA=0.019, 90% CI, 0.00-0.074). The Comparative Fit Index was 0.988. The CMIN/df was 1.053. The figures on the lines represent standardized beta values. The R^2 values were 15.3 % for IL-6, 12.8 % for CRP, 12.1 % for hepcidin, 7.4 % for ferritin. ** P<0.01, *P<0.05. Double headed arrows refer to inter-correlations. CRP, c-reactive protein; CMIN, chi-square; RMSEA, root mean square error of approximation; SLI, standard of living index.

for a period of 3 months in a sample of 233 and subsample of 80. Audio-visual aids including 2 pamphlets, 1 folder, one poster and an educational video (video studio software U Lead systems version 10.5 for 8 minutes in three languages, English, Telugu, Hindi) were used. Blood samples were collected and behavioural measures were recorded during the same period as baseline. Paired analysis was carried out for the changes in knowledge, micronutrient status, stress an allostatic load. The compliance for the nutrition education was >65%. There was a significant improvement in knowledge scores (P<0.001, figure 2). The micronutrients which showed an improvement were retinol and ascorbic acid. Vitamin B-12, alpha tocopherol and ferritin showed a decline. There was an increment in haemoglobin which may be on account of improvement in haemopoietic nutrient resulting in better mobilization of iron from stores. The relationship between retinol and knowledge persisted with an increment in both the variables within 4 months which signifies a proportionate increase which could be attributed to the positive effect of nutrition education.

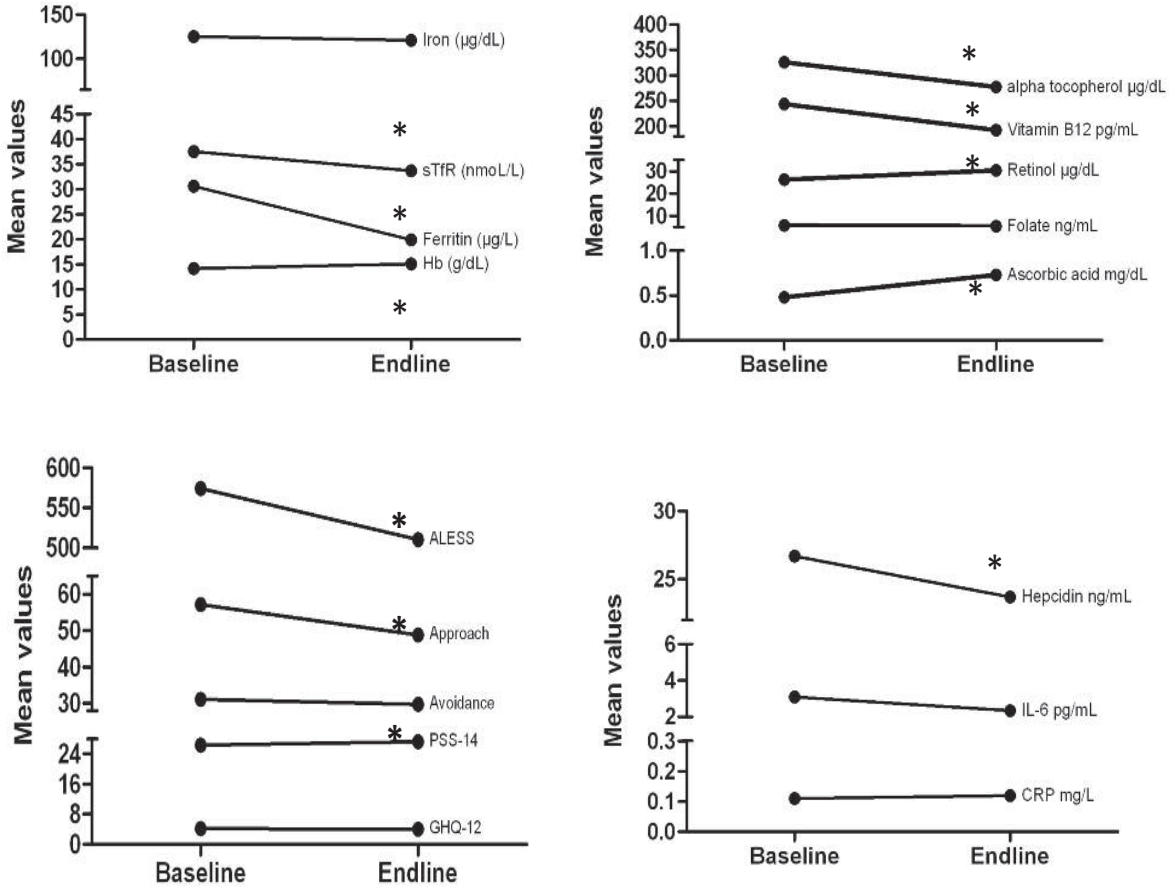
Figure 2. Distribution of knowledge scores before and after nutrition education. The median showed a shift from 5.0 to 7.0.



The inflammatory markers did not change while there was a decline in hepcidin. The reduction in hepcidin strengthens the earlier assumption that iron deficiency could be over-riding the inflammation mediated hypoferraemia. The possible effect of testosterone could not be ruled out in suppression of hepcidin. Perceived stress increased significantly over 1 year. Approach coping

declined. However, there was a reduction in the stressful life events. Individual variables of allostatic load showed a significant increase with respect to DHEAS, blood pressure, lipid profile and BMI. However allostatic load scores did not show any change over time (Figure 3).

Figure 3. Changes in variables after one year. Paired means were compared and P<0.05 was considered as significant difference.



* Represent significant difference between mean values. The dots represent means and the line represents the direction of change. Panel A, Iron status, Panel B. other micronutrients; Panel C. Stress variables; Panel D. IL-6- hepcidin axis.

Table 1. Relationship between coping strategies and vitamin B_{12}

	Unstandardized B coefficients	SE	Standardized B coefficients	t	P
Approach	1.7	0.57	0.243	2.94	0.004
BMI	-8.9	4.19	-0.175	-2.12	0.036

Stepwise regression, R^2= 7.7%, P= 0.004. Constant= 308.5. Other variables included were the scores on avoidance coping, SLI and haemoglobin. B, beta

CONCLUSIONS

- One third of the participants had allostatic load. The index showed a positive association with controllable life events of adolescent life event stress scale.
- Psychological stress was associated with elevated concentrations of inflammatory markers and

hepcidin but not leading to hypoferraemia. This may be due to the influence of various factors including multiple micronutrient deficiencies.

- Vitamin B-12 was positively associated with adaptive coping which needed to be probed further.
- Even though there was an improvement in knowledge on micronutrients after intervention, only ascorbic acid and retinol status improved while vitamin B-12 and ferritin concentrations declined, but there was no reduction in allostatic load or stress.

Thus, the study demonstrated that high psychological stress lead to high allostatic load. There was an association between iron status markers (hepcidin) and stress; vitamin B-12 with coping. Changes in certain micronutrients did not impact stress or allostatic load.

4 ASSESSMENT AND VALIDATION OF BODY COMPOSITION USING DIFFERENT TECHNIQUES AND DEVELOPMENT OF REGRESSION MODELS IN INDIAN POPULATION

The fat content of the human body has physiological, nutritional and medical importance; both in health and disease situations and hence there has been considerable interest in the measurement of human body composition in vivo over the past 4 to 5 decades. Several laboratories based techniques such as hydro- densitometry (under water measurement); measurement of naturally occurring ^{40}K isotope, bioelectrical impedance analysis and isotopic dilution technique using D_2O has been developed. All these techniques work on different principles and have the limitations such as being complex, expensive or time consuming.

The body size and composition of Indian population vary widely and differ markedly from those of Western population. Therefore, the usage of various regression equations to predict body composition that are developed on western population may not give accurate measurement of body composition in our population, since the regression equations would best fit in that populations where they are derived. This is because those factors are dependent upon age, sex and ethnicity. Hence, the present study is designed to validate the existing methods to assess body composition and to develop regression equations using skin fold measurement and bio-electrical impedance analysis methods to suite Indian population for accurate appraisal of body composition.

Therefore, keeping in view of the need of the day, to facilitate accurate and expedient method to measure body composition suitable to Indian population, the present study is proposed with the following objectives.

OBJECTIVES

- To examine the existing techniques used in the assessment of body composition and to identify the degree of agreement and suitability of different methods to the Indian population for accurate appraisal of body composition.
- To develop age and sex dependent regression equations for predicting body composition by using skin fold thickness measurement and bio-electrical impedance methods suitable to Indian population.

- To verify the degree of association of fatness (as assessed by hydro-densitometry) with body mass index (BMI) and Waist Hip Ratio (WHR) and to redefine the cut-off points suitable to our population.

RESULTS

A total of 1789 of which 766 males and 1023 females were screened for the study. The screening was continued till sufficient number of eligible participants agreed to participate in the study. About 483 subjects participated who met the eligibility criteria and who consented for the study, they consisted of 233 males and 250 females. The anthropometric measurements and body composition assessment using the four techniques as indicated in the methodology were carried out and the observations of the study are shown in table 1.

The mean values of physical characteristics like age, height, weight, BMI and Body Surface Area (BSA) of the subjects are given in the Table 2 and 3.

Table 1. Subject distribution

Category	Age Groups (years)	No. of subjects screened		No. of subjects participated	
		Males	Females	Males	Females
Pre-adolescent	7-10	147	211	55	60
Adolescent	10-18	280	271	56	60
Young adults	18-30	166	206	65	65
Adults	30-60	173	335	57	65
Total no: of subjects		766	1023	233	250
		1789		483	

Table 2. Physical characteristics of the male participants

Category	N	Age y	Height cm	Weight kg	BMI kg/m^2	BSA m^2
Pre-adolescents	55	8.8±0.9	124.9±6.9	22.9±3.3	14.6±0.9	0.899±0.089
Adolescents	56	14.8±2.2	160.3 ± 10.9	50.4±10.7	19.4±2.5	1.505±0.207
Young adults	65	24.5±3.2	168.0±5.5	60.3±6.3	21.3±1.8	1.683±0.103
Adults	57	42.6±9.2	165.8±6.4	62.1±6.0	22.6±1.7	1.688±0.107

In males, the mean age of the pre-adolescents, adolescents, young adults and adults were 8.8±0.9, 14.8±2.2, 24.5±3.2 and 42.6±9.2 years respectively. The height was found to be 124.9±6.9, 160.3±10.9, 168.0±5.5 and 165.8 ±6.4 cm and weight was found to be 22.9±3.3, 50.4±10.7, 60.3±6.3 and 62.1±6.0 kg for the pre-adolescents, adolescents, young adults and adults respectively. The BMI (kg/m^2) and BSA (sqm) respectively were found to be 14.6±0.9 and 0.899±0.089 in pre-adolescents, 19.4±2.5 and 1.505±0.207 in adolescents, 21.3 ± 1.8 and 1.683 ± 0.103 in young adults and 22.6±1.7 and 1.688±0.107 in adults.

Body Composition Analysis

The results of body composition analysis using different techniques are discussed below.

Body composition assessment using Hydro-densitometry

Hydro-densitometry was done for the subjects of the three age groups and the body composition parameters like lean body mass and fat mass were measured and the values are given in tables 4 and 5.

Table 3. Physical characteristics of the females participants

Category	N	Age y	Height cm	Weight kg	BMI kg/m^2	BSA m^2
Pre-adolescents	60	8.5 ± 0.8	122.5±7.5	21.3±3.4	14.1±1.0	0.860±0.094
Adolescents	60	14.5 ± 2.5	149.6±9.2	43.4±10.3	19.1±2.7	1.341±0.190
Young adults	65	22.9 ± 3.1	156.7±6.5	51.8±6.0	21.1±1.9	1.499±0.107
Adults	65	42.2 ± 8.7	155.2±5.3	55.1±5.9	22.8±2.1	1.529±0.096

In females, the mean age of the pre-adolescents, adolescents, young adults and adults were 8.5±0.8, 14.5±2.5, 22.9±3.1 and 42.2±8.7 years respectively. The height was found to be 122.5±7.5, 149.6±9.2, 156.7±6.5 and 155.2±5.3 cm and weight was found to be 21.3±3.4, 43.4±10.3, 51.8±6.0 and 55.1±5.9 kg for the pre-adolescents, adolescents, young adults and adults respectively. The BMI (kg/m^2) and BSA (sqm) respectively were found to be 14.1±1.0 and 0.860±0.094 in pre-adolescents, 19.1±2.7 and 1.341±0.190 in adolescents, 21.1 ± 1.9 and 1.499 ± 0.107 in young adults and 22.8±2.2 and 1.529±0.096 in adults.

Table 4. Body composition using hydro-densitometry in males (n=177)

Category	Density kg/L	Fat%	Fat kg	LBM kg
Adolescents	1.061±0.017	16.7±7.6	8.7±4.8	42.0±8.5
Young adults	1.063±0.014	15.6±6.1	9.6±4.2	50.7±5.0
Adults	1.049±0.011	22.1±4.9	13.8±3.8	48.2±4.3

In males, when body composition was measured using UWW method, the density was found to be 1.061±0.017, 1.063±0.014 and 1.049±0.011 respectively. The fat percentage was found to be 16.7±7.6, 15.6±6.1 and 22.1±4.9 respectively for the three groups studied. The LBM (kg) was found to be 42.0±8.5, 50.7±5.0 and 48.2±4.3 respectively.

Table 5. Body composition using hydro-densitometry in females (n=98)

Category	Density kg/L	Fat%	Fat kg	LBM kg
Adolescents	1.035±0.012	28.1±5.7	12.9±4.4	32.3±6.3
Young adults	1.037±0.009	27.6±4.2	14.3±3.3	37.1±3.9
Adults	1.036±0.006	27.8±2.5	14.4±2.8	37.4±6.3

Values are Mean±SD

In females, 98 subjects were covered using UWW method from three age groups as given in the Table-V. The density was found to be 1.035 ± 0.012, 1.037 ± 0.009 and 1.036 ± 0.006 respectively. The fat percentage was found to be 28.1 ± 5.7, 27.6 ± 4.2 and 27.8 ± 2.5 respectively for the three groups studied. On the other hand, their LBM (kg) was found to be 32.3 ± 6.3, 37.1 ± 3.9and 37.4 ± 6.3 respectively.

Body Composition Assessment using Air Displacement Plethysmography (ADP)

Air Displacement Plethysmography (ADP) was done to assess the body composition of the subjects in all the three groups mentioned. The body composition parameters are given in the Table 6 and 7.

Table 6. Body composition using ADP in males

Category	Volume L	Density kg/L	Fat%	Fat kg	LBM kg
Pre-adolescents	21.9±3.3	1.052±0.011	20.8±5.1	4.8±1.5	18.1±2.7
Adolescents	48.2±10.3	1.044±0.019	24.2±8.9	12.1±5.3	38.2±9.5
Young adults	57.3±6.3	1.053±0.014	20.1±6.2	12.3±4.4	48.0±4.8
Adults	59.7±6.1	1.040±0.015	25.8±6.7	16.1±4.7	46.0±5.2

Values are Mean±SD

The body volume was assessed by ADP using BodPod and density was calculated. The body volume of the subjects was found to be about 22L in pre-adolescents, 48 L in adolescents, 57 L in young adults and 60L in adults. The density was found to be 1.052±0.011, 1.044±0.019, 1.053± 0.014 and 1.040±0.015 respectively for the four groups and the fat percentage was found to be 20.8±5.1, 24.2±8.9, 20.1±6.2 and 25.8±6.7. The LBM was found to be 18.1±2.7, 38.2±9.5, 48.0±4.8 and 46.0±5.2 respectively for the four groups.

Table 7. Body composition using ADP in females

Category	Volume L	Density kg/L	Fat%	Fat kg	LBM kg
Pre-adolescents	20.2 ±3.4	1.060 ±0.014	17.0 ±6.3	3.8 ±1.8	17.6 ±2.2
Adolescents	41.8 ±10.3	1.039 ±0.014	26.5 ±6.6	11.9 ±5.1	31.5 ±6.1
Young adults	50.1 ±6.1	1.034 ±0.014	28.6 ±6.3	15.0 ±4.2	36.8± 4.1
Adults	53.7 ±6.5	1.022 ±0.014	34.4 ±6.7	19.2 ±5.1	35.7 ±3.2

Values are Mean±SD

The body volume was assessed by Air Displacement Plethysmography using BodPod and density was calculated. The body volume of the subjects was found to be about 20L in pre-adolescents, 42 L in adolescents, 50 L in young adults and 54 L in adults. The density was found to be 1.0601 ± 0.0144, 1.0390 ± 0.0145, 1.0344 ± 0.0136 and 1.0220 ± 0.0144 respectively for the four groups and the fat percentage was found to be 17.0 ± 6.3, 26.5± 6.6, 28.6±6.3 and 34.4 ± 6.7. The LBM was found to be 17.6±2.2, 31.5 ± 6.1, 36.8 ± 4.1and 35.7 ± 3.2 respectively for the four groups.

Body Composition Assessment using Skin Fold Thickness Method

The four skin fold thickness measurements were taken at triceps, biceps, sub-scapular and supra-iliac sites to assess the body composition of the subjects in the three age groups. The mean values of the sum of skinfold thicknesses at four sites along with the density and body composition are given the tables 8 and 9.

Table 8. Body composition using skin fold thickness measurement in males

Category	S of SKF	Density kg/L	Fat%	Fat kg	LBM kg
Pre-adolescents	22.6±5.3	1.067±0.006	14.0±2.7	3.2±0.9	19.7±2.7
Adolescents	40.1±15.8	1.054±0.011	19.5±5.0	10.0±3.7	40.4±8.4
Young adults	45.7±13.5	1.059±0.008	17.3±3.7	10.6±3.0	49.7±4.3
Adults	52.8±13.6	1.044±0.009	24.2±4.2	15.2±3.5	46.9±3.9

Values are Mean±SD

In males, the sum of the four skin folds were found to be 22.6±5.3, 40.1±15.8, 45.7±13.5 and 52.8±13.6 respectively for the four groups and the body density was calculated using Durnin and Womersley's age and gender specific equations and the values being 1.067±0.006, 1.054±0.011, 1.059±0.008, and 1.044±0.009 respectively. The density values were used to calculate fat percentage and it was found to be 14.0±2.7, 19.5±5.0, 17.3±3.7 and 24.2±4.2 while the lean body mass was found to be 19.7±2.7, 40.4±8.4, 49.7±4.3 and 46.9±3.9 in the four groups respectively.

Table 9. Body composition using skin fold thickness measurement in females

Category	S of SKF	Density kg/L	Fat%	Fat kg	LBM kg
Pre-adolescents	28.2±6.5	1.051±0.006	21.1±2.6	4.5±1.2	16.8±2.3
Adolescents	51.2±16.3	1.038±0.008	27.0±3.7	12.0±4.2	31.4±6.3
Young adults	58.9±14.6	1.034±0.008	28.6±3.7	14.9±3.2	36.9±3.6
Adults	73.0±20.4	1.022±0.011	34.6±5.1	19.2±4.5	35.7±2.6

Values are Mean±SD

In females, the sum of the four skin folds were found to be 28.2 ± 6.5, 51.2 ± 16.3, 58.9 ± 14.6 and 73.0 ± 20.4 respectively for the four groups and the body density was calculated using Durnin and Womersley's age and gender specific equations and the values being 1.051±0.006, 1.038 ± 0.008, 1.034 ± 0.008, and 1.022 ± 0.011 respectively. The density values were used to calculate fat percentage and it was found to be 21.1±2.6, 27.0 ± 3.7, 28.6 ± 3.7 and 34.6 ± 5.1, while the lean body mass was found to be 16.8±2.3, 31.4± 6.3, 36.9 ± 3.6 and 35.7 ± 2.6 in the four groups respectively.

Body Composition Assessment using Bioelectrical Impedance Analysis

The total body water (TBW) was assessed using BIA method and the lean body mass was calculated using hydration constant, 0.73 put forth by Pace and Rathbun (1945). The Extra cellular water (ECW), Intra Cellular Water (ICW), TBW, lean body mass and fat mass assessed by BIA is given in the Table 10 and 11.

Table 10. Body composition using bioelectrical impedance analysis in males

Category	TBW L	Fat %	Fat kg	LBM kg
Pre-adolescents	14.4±1.8	14.2±4.1	3.3±1.3	19.6±2.5
Adolescents	29.7±6.7	19.6±7.4	10.0±4.6	40.4±9.1
Young adults	35.1±3.2	20.3±5.3	12.4±4.1	47.9±4.3
Adults	34.0±3.1	25.2±5.1	15.8±4.0	46.3±4.3

Values are Mean±SD

In males, the TBW was found to be 14.4±1.8, 29.7±6.7, 35.1±3.2 and 34.0±3.1 respectively for the four groups of females studied. The LBM was found to be 19.6±2.5, 40.4±9.1, 48.1±4.5 and 46.3±4.3 and the fat percentage was found to be 14.2±4.1, 19.6±7.4, 20.3±5.3 and 25.2±5.1 in the four groups respectively.

Table 11. Body composition using bioelectrical impedance analysis in females

Category	TBW L	Fat%	Fat kg	LBM kg
Pre-adolescents	12.8±1.8	17.8±4.6	3.9±1.4	17.5±2.4
Adolescents	21.9±4.3	29.1±7.4	13.2±6.0	30.2±5.1
Young adults	25.2±2.9	33.3±5.0	17.4±3.8	34.4±3.9
Adults	24.9±2.4	37.9±4.8	21.0±4.2	34.0±3.2

Values are Mean±SD

The TBW was found to be 12.8±1.8, 21.9 ± 4.3, 25.2 ± 2.9 and 24.9 ± 2.4 respectively for the four groups of females studied. The LBM was found to be 17.5±2.4, 30.2 ± 5.1, 34.4 ± 3.9 and 34.0 ± 3.2 and the fat percentage was found to be 17.8±4.6, 29.1 ± 7.4, 33.3 ± 5.0 and 37.9 ± 4.8 in the four groups respectively.

Table 12. Body composition profile of the males using different techniques

Age Group (yrs)	Weight (kg)	UWW (n= 177)		BodPod (n = 233)		SKF (n = 233)		BIA (n = 233)	
		Fat (%)	LBM (kg)	Fat (%)	LBM (kg)	Fat (%)	LBM (kg)	Fat (%)	LBM (kg)
Pre-adolescents (7-10)	22.9 ±3.3	-	-	20.8 ±5.1	18.1 ±2.7	14.0 ±2.7	19.7 ±2.7	14.2 ±4.1	19.6 ±2.5
Adolescents (10-18)	50.4 ±10.7	16.7 ±7.6	42.0 ±8.5	24.2 ±8.9	38.2 ±9.5	19.5 ±5.0	40.4 ±8.4	19.6 ±7.4	40.4 ±9.1
Young Adults (18-30)	60.3 ±6.3	15.6 ±6.1	50.7 ±5.0	20.1 ±6.2	48.0 ±4.8	17.3 ±3.7	49.7 ±4.3	20.3 ±5.3	48.1 ±4.5
Adults (30-60)	62.1 ±6.0	22.1 ±4.9	48.2 ±4.3	25.8 ±6.7	46.0 ±5.2	24.2 ±4.2	46.9 ±3.9	25.2 ±5.1	46.3 ±4.3

In males, the mean fat percentage assessed by four different methods varied from 16.7±7.6 to 24.2±8.9 for adolescents, 15.6±6.1 to 20.1±6.2 for young adults and 22.1±4.9 to 25.8±6.7 for adults. In pre-adolescents, the fat percentage measured using Bodpod, SKF and BIA was found to be between 14.0±2.7 and 20.8±5.1.

In females, the mean fat percentage assessed by four different methods varied from 26.5 ± 6.6 to 29.1 ± 7.4 for adolescents, 27.6 ± 4.2 to 33.3 ± 5.0 for young adults and 27.8 ± 2.5 to 37.8 ± 5.0 for adults. In pre-adolescents, the fat percentage measured using Bodpod, SKF and BIA was found to be between 17.0 ± 6.3 and 21.1±2.6. There was an increase in fat percentage with age from pre-adolescents (7-10yrs) to adolescents (10-18yrs), adolescents (10-18yrs) to young adults (18-30yrs) and young adults to adults (30-60yrs) except when measured by UWW where adolescents got higher mean of fat percentage than the two other groups.

Table 13. Body composition profile of the females using different techniques

Age Group (yrs)	Weight (kg)	UWW (n= 98)		BodPod (n = 251)		SKF (n = 251)		BIA (n = 250)	
		Fat (%)	LBM (kg)	Fat (%)	LBM (kg)	Fat (%)	LBM (kg)	Fat (%)	LBM (kg)
Pre-adolescents (7-10)	21.3			17	17.6	21.1	16.8	17.8	17.5
	±3.4	-	-	± 6.3	± 2.2	± 2.6	± 2.3	±4.6	±2.4
Adolescents (10-18)	43.4	28.1	32.3	26.5	31.5	27	31.4	29.1	30.2
	±10.3	±5.7	±6.3	±6.6	±6.1	±3.7	±6.3	±7.4	±5.1
Young Adults (18-30)	51.8	27.6	37.1	28.6	36.8	28.6	36.9	33.3	34.4
	±6.0	±4.2	±3.9	±6.3	±4.1	±3.7	±3.6	±5.0	±3.9
Adults (30-60)	55.1	27.8	38.6	34.4	35.8	34.7	35.7	37.9	34
	±5.9	±2.5	±5.9	±6.7	±3.2	±4.9	±2.7	±4.8	±3.2

Comparison of different methods in assessing body composition

The fat percentages measured by different body composition methods were compared with the reference method (either UWW or ADP method wherever applicable) using paired t-test, and the results are given in the Table 14.

Table 14. Comparison of fat percentage as measured by different methods

Age Group	Males				Females			
	UWW	ADP	SKF	BIA	UWW	ADP	SKF	BIA
Preadolescents (7-10)		20.8± 5.1*	14±2.7 (0.000)	14.2±4.1 (0.000)		17±6.3*	21.1±2.6 (0.000)	17.8±4.6 (0.109)
Adolescents (10-18)	16.7± 7.6*	24.2±8.9 (0.000)	19.5±5.0 (0.000)	19.6±7.4 (0.000)	28.1 ± 5.7*	26.5±6.6 (0.000)	27±3.7 (0.001)	29.1±7.4 (0.002)
Young adults (18-30)	15.6± 6.1*	20.1±6.2 (0.000)	17.3±3.7 (0.002)	20.3±5.3 (0.000)	27.6± 4.2*	28.6±6.3 (0.526)	28.6±3.7 (0.178)	33.3±5.0 (0.000)
Adults (30-60)	22.1± 4.9*	25.8±6.7 (0.000)	24.2±4.2 (0.000)	25.2±5.1 (0.000)	27.8± 2.5	34.4± 6.7*	34.7±4.9 (0.777)	37.9±4.8 (0.000)
Post menopausal						39.2± 4.3*	22.8±2.8 (0.830)	40.7±3.7 (0.025)

*Reference method, P values are given in parenthesis.

The fat percentage measured by different body composition methods were compared with the reference methods (either UWW or ADP), and the significance levels are given in the parenthesis. In males, UWW was considered as the reference method in adolescents, young adults and adults while ADP was considered as the reference method in pre-adolescents. There was a significant difference ($p<0.01$) between the fat percentage measured using reference method and that of other methods in all the age groups. Hence the methods were not comparable to the reference methods.

In females, the UWW was considered as the reference method in adolescents and young adults and ADP was considered as the reference method in pre-adolescents, adults and post menopausal women. Fat percentage assessed using SKF was comparable ($p>0.01$) in young adults, adults and postmenopausal group whereas, in pre-adolescents and adolescents it was not comparable ($p<0.001$) with that of reference methods. Fat percentage assessed by BIA was comparable in pre-adolescents ($p<0.01$) and post-menopausal ($p<0.05$) whereas in other age groups it was not comparable with that of any of the given reference methods.

5. AMINO ACID – METAL COMPLEXES AS MODEL FOR THE GLUCOSE TOLERANCE FACTOR OF YEAST: HYPOGLYCAEMIC ACTIVITY AND THERAPEUTIC POTENTIAL IN DIABETES; SYNTHESIS, STRUCTURE AND MECHANISM OF ACTION IN YEAST AND ANIMALS

Glucose Tolerance Factor (GTF), an ill-defined chromium containing complex with amino acids: phenylalanine, glutamic acid, glycine, cysteine, and Nicotinic acid, plays a role similar to that of insulin in mammals. When added exogenously to Yeast cultures, it enhances the rate of glucose metabolism, making yeast a model organism to study biological role of chromium. Many ambiguities regarding the actual structure, nature of binding between AA to Cr^{+3} and minimal ligand requirements regarding GTF still exist, since in the years after its discovery, several such complexes were isolated from varied sources with different AA ligands, but having similar activity. Attempts to synthesize soluble, stable chromium - amino Acid complexes have failed to date.

Most of the studies have done were included regular organic synthesis procedures, using high temperature, alkaline pH etc. Recently, a $(D\text{-}Phe)_3Cr$ was synthesized and was shown to have GTF like activity, but this too turned out to be insoluble. Present studies aim at understanding some of these aspects using Yeast (*S. cerevisiae* NCIM 3559) as a model organism for testing the effects of AA.Cr complexes on glucose uptake by yeast (including determination of initial kinetics of glucose utilization). Subsequently, those complexes which showed positive & negative results in the yeast were tested in animal models for their effects on oral glucose tolerance test (OGTT). For this purpose, a number of AA – Cr complexes were synthesized, purified and their physicochemical properties studied. Interestingly, similar activity and striking parallelism were found between the two systems in the effects of the AA – Cr complexes on glucose uptake / OGTT. These studies are the first of their kind and open a path for understanding the biological role of Cr^{+3} in yeast and animals.

OBJECTIVES

- Synthesize, purify and characterize AA - Cr complexes using minimal optimal conditions.
- Screen synthetic AA – Cr complexes in the yeast (*S cerevisiae*) system to identify the most active ones having GTF like activity and test the effects of these compounds with GTF like activity in the yeast system for their effects if any on the OGTT in rat models. Assess how their activity compares with that of isolated GTF from yeast and LMWCr.
- Examine the mechanism of action of different AA- Cr complexes found active in the yeast and experimental animals, especially those of relevance to known metabolic changes associated with glucose metabolism.

- Assess the possible role of additional ligands apart from Amino acids in complexes of the type $(AA)_{nCr.x}$.
- Elucidate the structures and physico-chemical properties of different complexes synthesized and examine whether other AA - Cr complexes exhibit similar properties like those of AA – Cr complexes.

WORK DONE

- During Last year various chromium –amino acid complexes and screened their effect in type I diabetic (streptozotocin induced) animal models were synthesized. Among these only chromium-phenyl alanine complex showed significant effect as hypoglycaemic agent that too in only in diabetic animals. Considering that type 2 diabetes is the more common and predominant form, the effects of the chromium amino acid complexes in the animal models of insulin resistance / type 2 Diabetes had assessed.
- During this year animal models with insulin resistance by feeding with high sucrose diet for 12 weeks were developed. After conforming that the animals were insulin resistant, they were divided into different groups and treated with complexes of different amino acids to assess their anti hyperglycaemic effects.

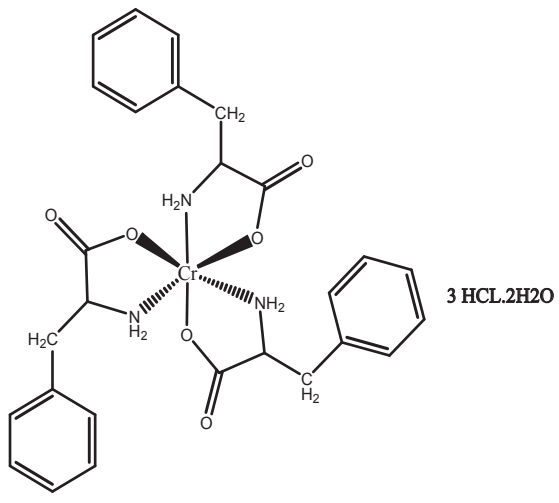
RESULTS

Synthesis of Cr (L-phe)$_3$: Cr (L-phe)$_3$ was synthesized and characterized as described previously (16). Briefly, aqueous solutions (50 mL each) of $CrCl_3$ $6H_2O$ (2.6 g, 10 mmol) and L-phenylalanine (4.8 g, 30 mmol) were mixed at 80°C and refluxed for 4 h. The homogeneous green reaction mixture was freeze-dried. The greenish-violet solid obtained was washed with acetone and dried in an air oven. The remain amino acid with chromium complex was obtained by a similar procedure to that described above.

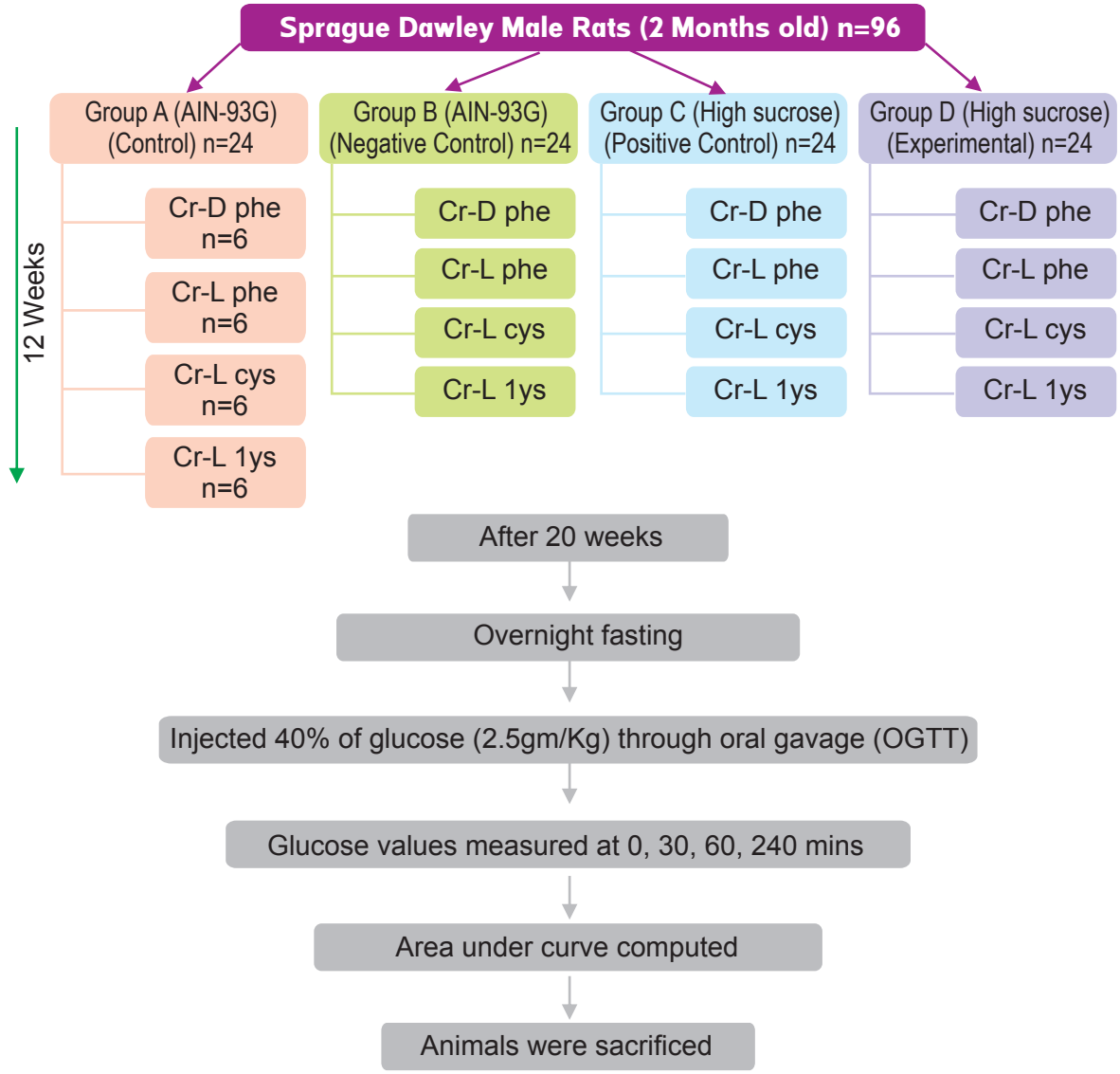
Physicochemical characterisation of AA – Cr complexes: For physicochemical characterization, the purified complexes were subjected to spectral studies in the UV/Vis region and typical example of Cr-Phe is shown Figure 2.The product obtained using the method has several unique properties. It exists as a stable solid. It is dark purple in colour. The product is readily soluble in water and methanol, soluble in ethanol, sparingly soluble in isopropyl alcohol and insoluble in ethyl acetate. A 0.1molar solution of the product in water has a pH of 4.1. The UV/Vis spectrum of the solution has absorption maxima at 400 nm (molar absorptovity, 44.08) and at 541 nm (molar absorptivity, 50.60) in contrast; the UV/Vis spectrum of chromium chloride has absorption maxima at 429 nm (molar absorptivity18.10) and at 608 nm (molar absorptivity, 14.43).

The addition of 20 molar equivalents of a 0.1 molar solution of sodium bicarbonate changed the pH of the solution to 8.1 but no precipitate was formed. Complexes with similar physicochemical properties were obtained using different alpha amino acids. Elementary analysis of the complexes gave results consistent with the proposed structure.

Figure 1. Chemical structure of Cr (AA)$_3$



a. Animal Experiment



b. Oral Glucose Tolerance Test (OGTT):

Considering that some earlier studies had demonstrated the beneficial effects on OGTT of Cr (in complex form) when given at a conc. of 45 µg/kg body weight, asimilar dose of the AA-Cr complexes were used in the present study to assess their anti hyperglycaemic effects.

Following an overnight fast and subsequent to blood withdrawal from supra orbital sinus the animals were administered a glucose solution (40 %) through an oral gavage at the rate of 2.5 gm glucose/ kg body weight. Blood glucose concentrations were tested subsequently by the tail vein prick method at 0, 30, 60, and 120 minutes following the glucose load using a glucometer (Rite Check Blood Glucose Monitoring System, Manufactured by: OK Biotech Co.,Ltd., Taiwan. Imported and Marketed by MedPlus Health Service Pvt.Ltd, # 12-7-20/54/2, Goods Shed Road, Moosapet, Hyderabad - 500018). To test the effects of Cr.AA complexes, they were administered to rats along with the glucose solution at a conc. of 45 g/kg body weight. AUC values are shown in Table 4 and means were compared between control and experimental groups.

c) Tables and Figures

Figure 1. Uv/VisSpectra of Different Cr.AA complexes

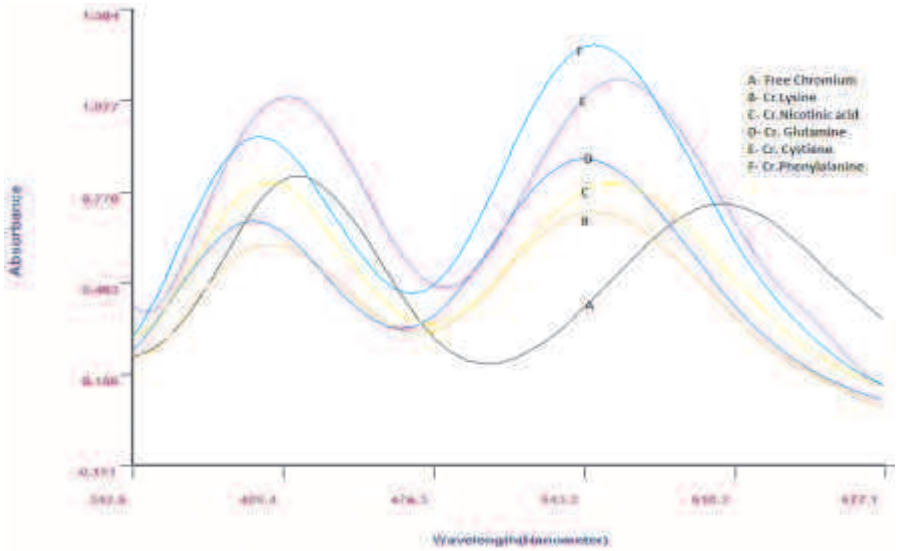


Table 1. UV/Vis Spectra of Various Cr.AA Complexes in nms

Cr.AA Complexes	max(A)	max(B)	(A)	(B)
Free Chromium	417	602	------	------
Phenylalanine	400	547	17	49
Lysine	412	558	05	44
Cystine	402	549	15	53
Glutamine	403	543	14	59
Nicotinic Acid	401	552	16	50

Difference in -max Indicates that complexation may takesplace.

Figure 2. FTIR of Cr.AA Complexes (1:3)

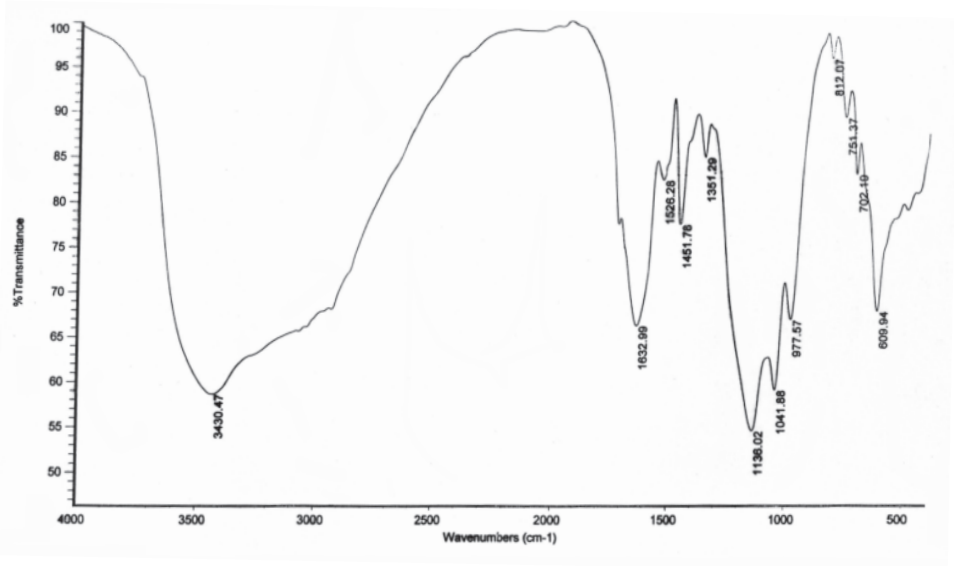


Table 2. FTIR Spectra of Cr.AA Complexes (Strong peaks) in cm^{-1}

Cr.AA Complexes	N-H (3400-3250)	C-H (3100-2700)	C=O (1760-1600)	C-O (1350-1000)	C-N (1280-1050)	Cr-O (540-450)	Cr-N (500-420)	S-H (1670-1500)
Free-(L)Phe ala	3400	2875	1610	1315	1225	--	--	--
Cr-(L)Phenylalanine	3407	2883	1615	1319	1261	458	453	---
Free-(D)Phenylalanine	3405	2875	1610	1317	1228	--	--	--
Cr-(D)Phenylalanine	3410	2886	1620	1325	1267	460	455	--
Free Lysine	3380	2868	1625	1328	1250	--	--	--
Cr-Lysine	3385	2871	1628	1336	1256	462	439	---
Free Cystine	3420	2920	1615	1343	1210			
Cr-Cystine	3422	2923	1618	1346	1213	453	442	1575

In FTIR Strong Cr-O, Cr-N Peaks indicate complex formation between CR and the amino acid .

Table 3. Glucose Area under curve (m mol.min/L) for various Cr.AA complexes in Sprague Dawley male rats

S.No	Cr.AA complex	C	C+	S	S+
1	L-phe	769 ± 9.65	819 ± 6.52	1293 ± 5.21	965 ± 1.25*
2	D-phe	"	829 ± 10.2	"	954 ± 12.8 *
3	L-cys	"	816 ± 11.0	"	1213 ± 11.0
4	L-lys	"	896 ± 5.29	"	1176 ± 10.2
5	1:1:1	"	878 ± 9.25	"	1037 ± 5.21

C Control;C+ negative control;S positive Control; S+ Experimental;
Values are mean ± SE (n=6). * Significantly different at p 0.05 using student t test

Table 4. Insulin Area under curve (p mol/L) for various Cr.AA complexes in Sprague Dawley male rats

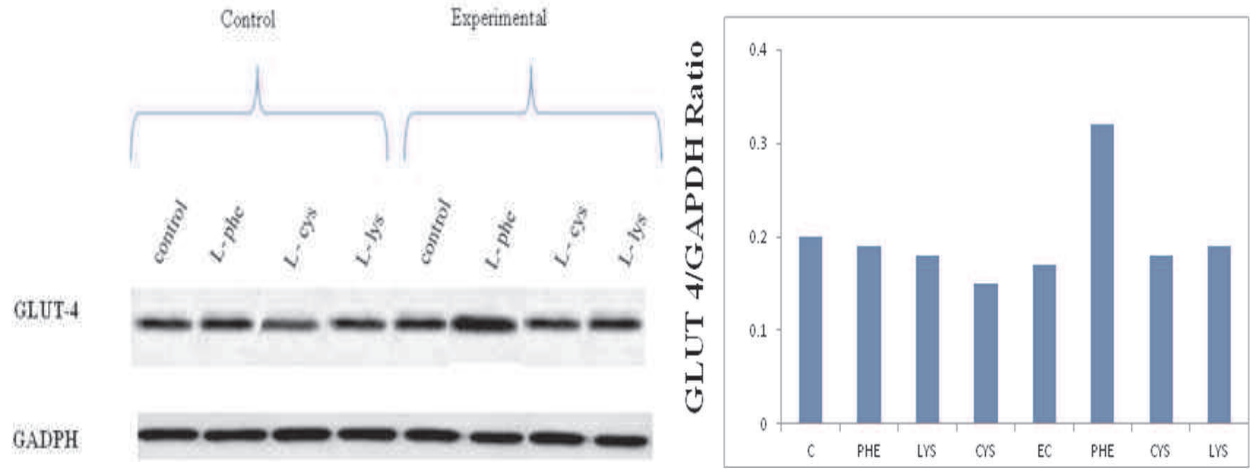
S.No	Cr.AA complex	C	C+	S	S+
1	L-phe	138 ± 21	142 ± 32	257 ± 26	234 ± 28
2	D-phe	"	137 ± 18	"	238 ± 32
3	L-cys	"	148 ± 20	"	246 ± 16
4	L-lys	"	130 ± 22	"	238 ± 18
5	1:1:1	"	123 ± 19	"	218 ± 36

C Control;C+ negative control;S positive Control; S+ Experimental;
Values are mean ± SE (n=6). * Significantly different at p 0.05 using student t test

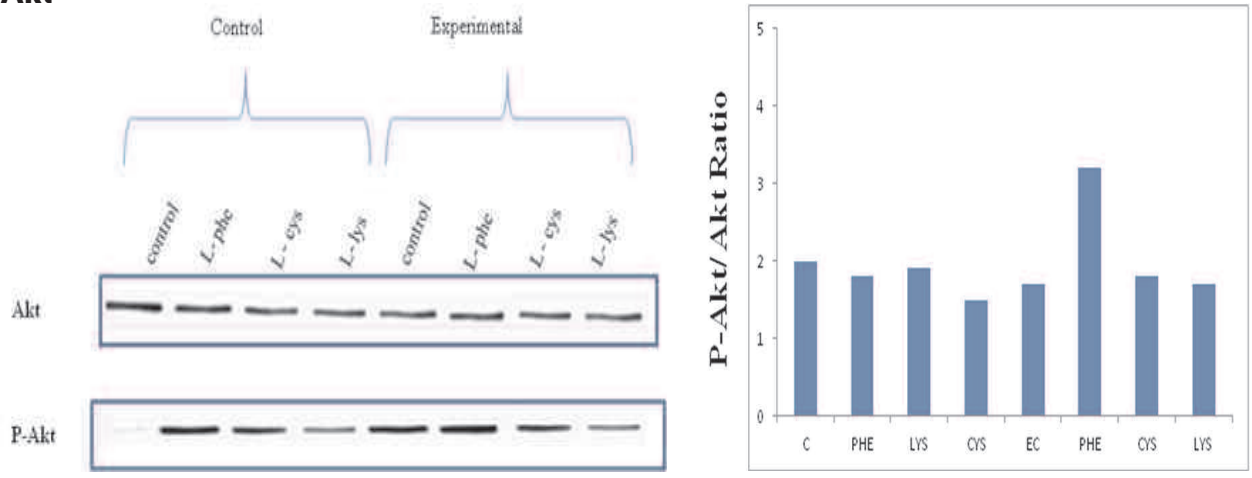
b) Western-blot analysis:

Skeletal muscle was rapidly dissected, homogenized, and subjected to Western blot analysis. The intensity of bands was measured with a scanning densitometer (Model GS-800; Bio-Rad) coupled with Bio-Rad PC analysis software.

GLUT 4



Akt



The mechanism by which chromium imparts its beneficial effects is yet unclear, although several hypotheses have been proposed. The biologically active chromium complex, Chromodulin, has been shown to augment kinase activity of the insulin receptor in adipocytes. The major finding from the present study is that chronic oral supplementation with Cr (phe) 3 improved glucose tolerance in a dietary, sucrose-fed mouse model of insulin resistance. Skeletal muscle from Cr (D-phe) 3-treated mice had enhanced Akt-phosphorylation and membrane translocation of GLUT4.

The results from this study show that a chromium Phe complex alleviates diet-induced insulin resistance, which may be mediated through insulin signaling. These results suggest that nutritional supplementation with chromium complexes may have potential therapeutic value in treating or preventing insulin resistance associated with metabolic syndrome.

6 IGF1 AND BDNF SIGNALING IN THE BRAIN OF WISTAR NIN OBESE MUTANT RATS DURING AGEING: EFFECT OF CALORIE AND MICRO-NUTRIENT RESTRICTIONS

Insulin-like Growth Factor (IGF1) is an anabolic and neuroprotective factor which promotes neuronal survival by blocking apoptosis. However, studies show that an excess of IGF1 may trigger neurodegeneration. Increasing evidence implicates brain IGF1 signaling in life-span control and it is shown that this function is conserved throughout evolution. Similarities in longevity regulatory pathways from yeast to mice suggest that insulin / IGF-1 signaling pathways may also regulate cell damage and longevity in humans. Calorie restriction extends longevity in many organisms and BDNF signaling is known to be involved in this effect of calorie restriction. Interestingly, life-span regulation by insulin-like metabolic control is analogous to mammalian longevity enhancement induced by calorie restriction, suggesting a general link between metabolism and life span of organisms.

WNIN obese mutant rat models (WNIN-Ob and WNIN – GR/Ob) developed at the NCLAS, NIN, Hyderabad from the Wistar/ NIN rat colony have higher oxidative stress than controls of comparable age and show accelerated ageing / reduced longevity. However, the exact biochemical /molecular basis of accelerated ageing in WNIN obese mutant rats remain to be deciphered. The present studies are conducted to validate/ negate the hypothesis that altered IGF1 and / or BDNF signaling in brain could underlie accelerated ageing in NIN obese mutant rats.

OBJECTIVES

- To evaluate whether alterations in IGF1 and BDNF signaling in the brain underlie/ are associated with accelerated ageing observed in the NIN obese mutant rat strains compared to WNIN control rats.
- To determine the effects of calorie and/or micro-nutrient restrictions on brain IGF 1/BDNF signaling and oxidative stress in NIN obese mutant rat strains *vis a vis* accelerated ageing observed in them.

RESULTS

1. Circulating and central levels of IGF1, BDNF and pituitary hormones

Peripheral blood, hypothalamus, cerebral cortex and CSF were collected from six months old rats of WNIN – Ob and WNIN strains (n= at least six for each strain). In an attempt to assess whether WNIN – Ob rats differ from WNIN controls in their levels of IGF1 and BDNF, the concentrations of IGF1, BDNF and other pituitary hormones were analyzed in plasma by separate Milliplex MAP kits for IGF1 and Rat Pituitary Hormones (Millipore Corporation, USA) as per the manufacturer's instructions.

The data on the plasma profile of IGF1, BDNF, GH, TSH, ACTH, LH, FSH and prolactin in six months old WNIN and WNIN – Ob mutant rats (n = 6 each) is given in Figure 1. They indicate that obese mutants had higher levels of IGF 1 (albeit not significant) and significantly lower levels of BDNF than WNIN controls. While prolactin levels were significantly higher in obese mutants than controls, GH levels were significantly lower albeit in only males. On the other hand levels of ACTH, LH and FSH showed variations among males and females whereas TSH levels were comparable among the genders. Taken together with significantly higher oxidative stress demonstrated earlier in WNIN – Ob mutant rats than WNIN controls of corresponding age, the present results appear to suggest that modulation of IGF1 and BDNF signaling could also underlie / be associated with

accelerated ageing in WNIN -Ob mutant rats. However, these findings need to be supplemented by determining the levels and / or activity status of these molecules in hypothalamus, cerebral cortex and CSF of rats.

Figure 1: Quantification of (A) IGF1, (B) BDNF, (C) GH, (D) TSH, (E) ACTH, (F) LH, (G) Prolactin and (H) FSH in the plasma samples from the male and female groups of 6 month old WNIN control and WNIN Obese mutant rats

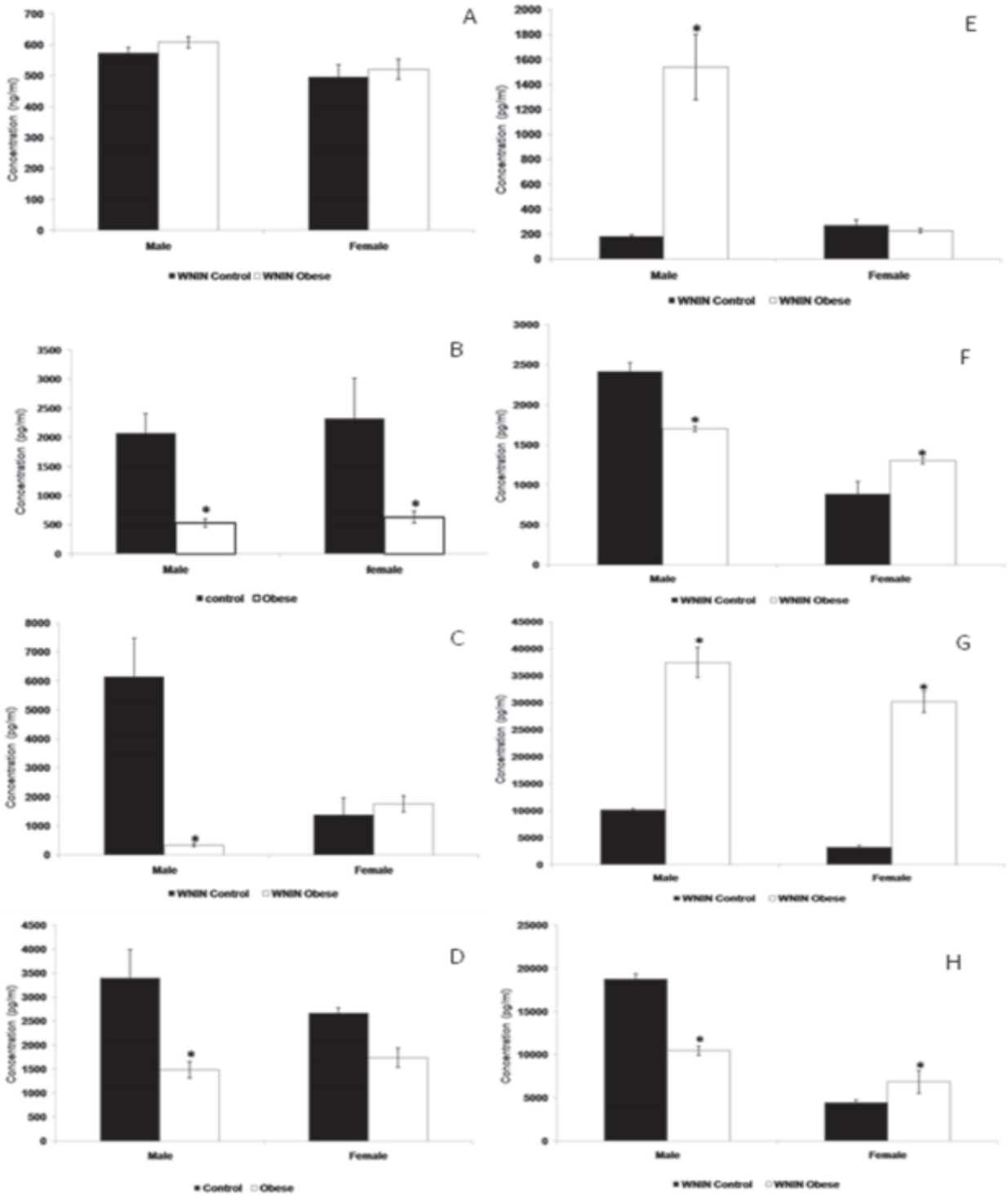


Table: Plasma levels of various growth factors and pituitary hormones in the male and female groups of 6 month old WNIN control and WNIN Obese mutant rats

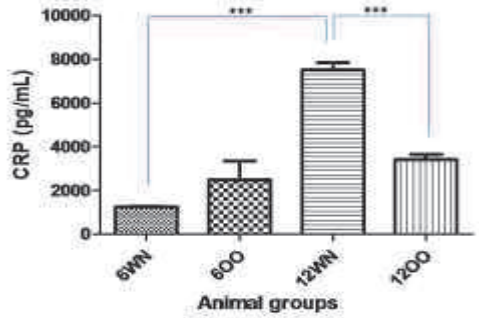
Growth factor/ hormone	Units	Male		Female	
		WNIN Control	WNIN Obese Mutant	WNIN Control	WNIN Obese Mutant
IGF-1	ng/ml	574 - 17.5	609 - 17.8	497 - 39.2	521 - 32.4
BDNF	pg/ml	2070 - 337*	529 - 69.6*	2325 - 691*	630 - 99.4
GH	pg/ml	6153 - 1319*	342 - 56.1*	1383 - 591	1758 - 281
TSH	pg/ml	1485 - 112	1738 - 197	3404 - 591	2667 - 174
ACTH	pg/ml	182 - 9.47*	1540 - 261*	272 - 42.5	226 - 15.4
LH	pg/ml	2417 - 107*	1702 - 159*	884 - 31.4*	1304 - 14.3*
Prolactin	pg/ml	10198 - 214*	37474 - 2828*	3247 - 301*	30160 - 1969*
FSH	pg/ml	18802 - 574*	10473 - 550*	4462 - 325*	7944 - 1483

Values are mean ± SE (n=6).
Means with a star (*) mark are significantly different at p<0.05 by independent t-test

1. C-reactive protein (CRP) levels in the plasma in the WNIN Ob mutants rats as compared to the WNIN control rats of 6 and 12 months age.

CRP is a marker of systemic low-grade inflammation, and has been associated with androgen deficiency and ageing. CRP elevation can be caused by conditions other than inflammation and may reflect biologic ageing. Changes (albeit not significant at 6 months of age but significant in 12-13 months of age : p<0.0001) were observed in the levels of CRP in the plasma of the WNIN Ob mutant rats as compared to the age-matched WNIN control. The CRP levels were significantly enhanced at 12 months of age in the normal WNIN rats but not in the WNIN/Ob rats which may reflect the compensatory mechanisms to counteract the complex altered physiologic conditions of the WNIN/Ob rats.

Figure 1. CRP levels in the plasma of WNIN/Ob and WNIN rats at two time points
(6 & 12 months)



Each bar represents a mean ± SE (n=6). There is a significant increase (p<0.0001) by one way ANOVA followed by post hoc test in normal (WN) rats at 12 months of age as compared to the 6 months WN rats. On the other hand, there is a significant decrease (p<0.0001) of CRP levels in 12 months old WNIN/Ob rats as compared to age matched WNIN rats.

2. Glial fibrillary acidic protein (GFAP) in the brain of WNIN Ob mutants rats as compared to WNIN control using immunohistochemistry.

Glial activation, in particular the increased expression of GFAP, may be a factor in impaired synaptic plasticity which is further related to normal brain ageing. Both protein and mRNA levels of GFAP have shown to increase with age and neurodegeneration. Increased expression of GFAP was found in the hippocampus of 6 months old WNIN Ob mutants rats as compared to age-matched WNIN control rats. But surprisingly, may be as a compensatory mechanism the expression was found to be less at 12-13 months of age in the WNIN/Ob rats.

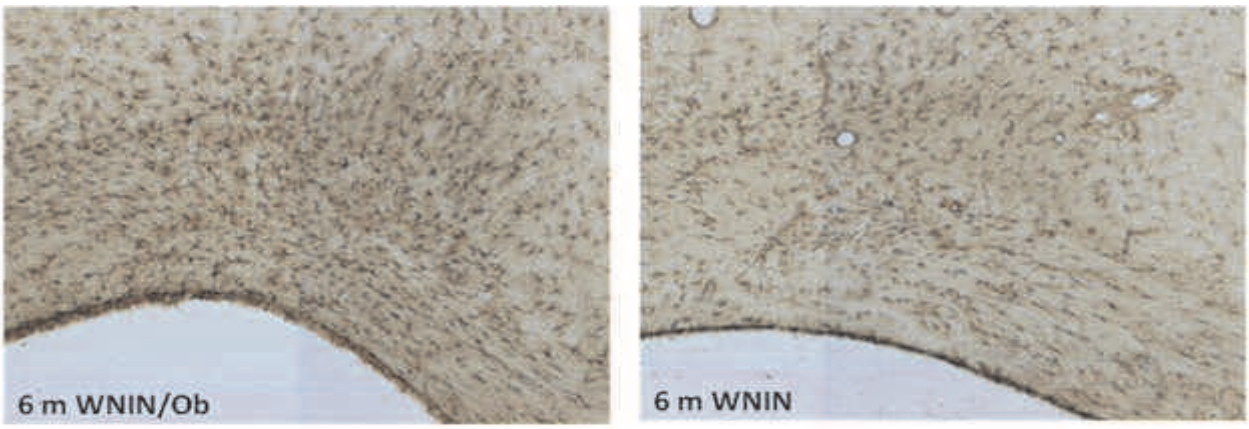


Fig. 2: Comparative images (4X) for increased GFAP expression as well as high GFAP positive cells in the hippocampus of 6 months old WNIN/Ob and WNIN normal control rats

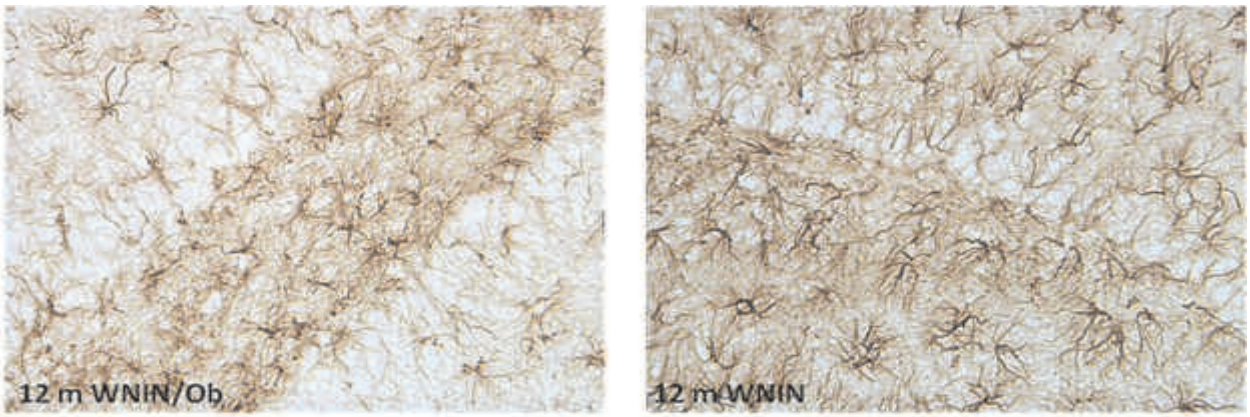


Fig. 3: Comparative images (20X) for GFAP expression in the hippocampus of 12 months old WNIN/Ob and WNIN normal control rats. Due to ageing the 12m WNIN shows increased proliferation of astrocytes but in WNIN/Ob suppressed astrocytic activity can be seen as a compensatory mechanism.

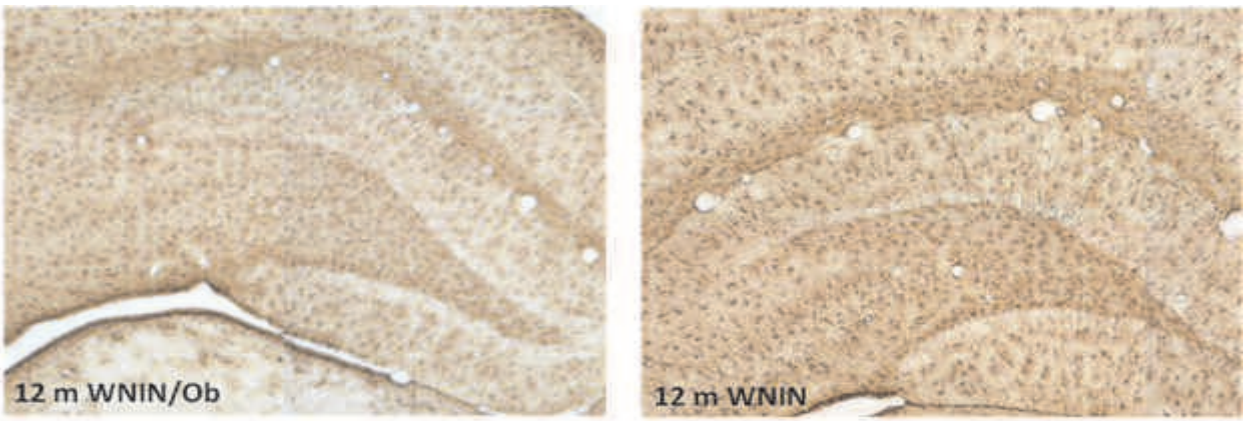


Fig. 4: Comparative images (4X) for GFAP expression in the hippocampus of 12 months old WNIN/Ob and WNIN normal control rats. Due to ageing the 12m WNIN rats show increased proliferation of astrocytes but in WNIN/Ob suppressed astrocytic activity can be seen as a compensatory mechanism.

3. Qualitative and quantitative (cell counts) evaluation of orexinergic neurons in the brain of WNIN Ob mutants rats as compared to WNIN control using immunohistochemistry for orexin-A (ORX-A). (Data analysis through Image ProPlus 7.0 software for quantitative image analyses of immunolabeling)

Orexin/ hypocretin is indicated to affect various physiological functions and behaviors, such as energy balance, feeding, wake–sleep cycle, stress response and reproduction. An increased protein expression of ORX-A as well as the ORX-A positive neuronal count (significantly high; $p<0.001$) were found in the brain of 12 – 13 months old WNIN Ob mutants rats as compared to age-matched WNIN control.

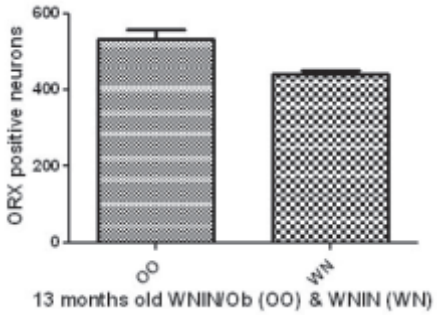
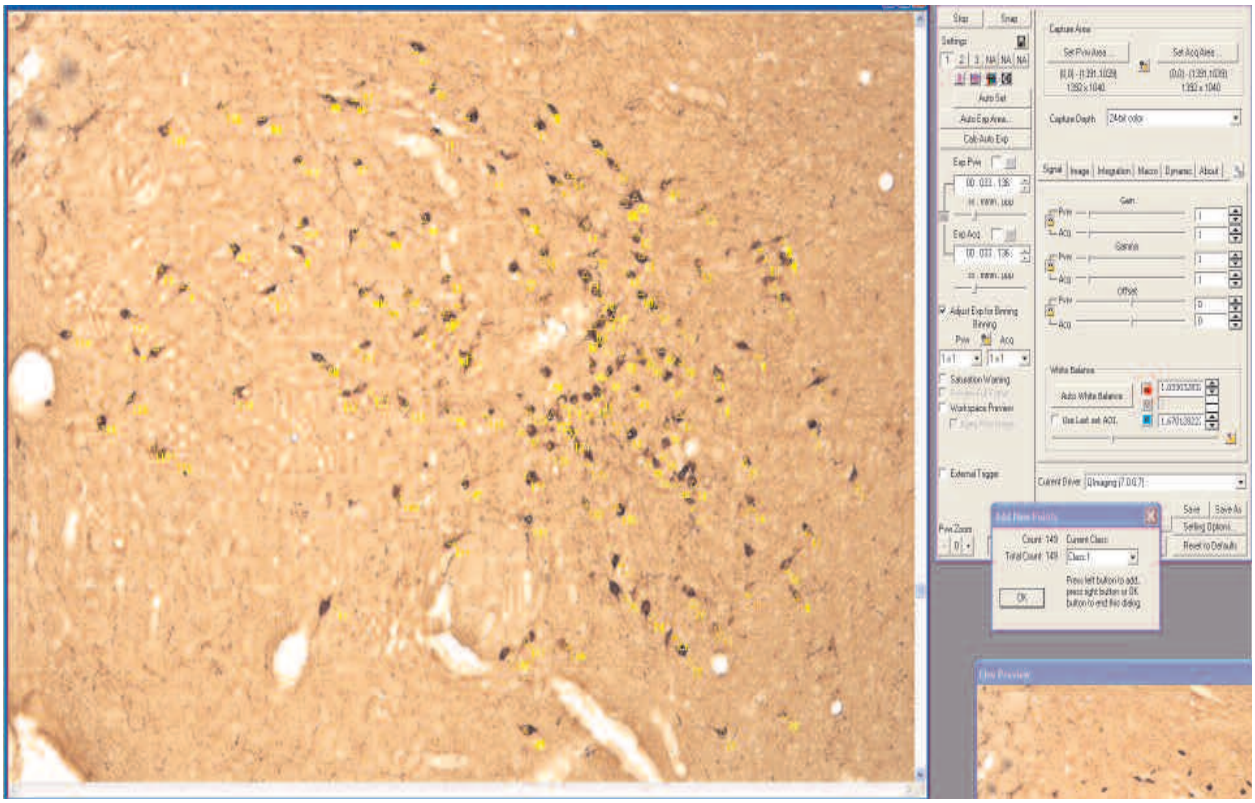


Figure 5. Counts of ORX-immunolabeled cells in the posterior lateral and dorsomedial areas of hypothalamus.
The bars represent a mean ± SE (n=6) at the significance level of $p<0.05$ using unpaired t-test. Counting was performed in each hemisphere using two adjacent frames which were enough to include all the ORX+ cell bodies in the hemisphere.

The mean number of ORX-immunolabeled cells in the posterior lateral and dorsomedial areas of hypothalamus of the 13 months old WNIN/Ob rats was 533.2 ± 24.78 (n=6) where as in the WNIN control rats it was 441.2 ± 9.796 (n=6).

Figure 6. Manual counting of ORX-immunolabeled cells in the posterior lateral and dorsomedial areas of hypothalamus using a 4X objective and the image analysis system ImagePro Plus 7.5 (Media Cybernetics, Silver Spring, MD)



7 FUNCTIONAL ASSESSMENT OF ADULT HUMAN PANCREATIC ISLETS FOLLOWING AUTOLOGUS TRANSPLANTATION

Islet cell transplantation appears to be an attractive option over exogenous insulin therapy in the treatment of diabetes (type 1 and secondary diabetes) since the transplanted islets respond to physiological needs of the body. However, inherent problems such as intense immuno-suppression, lack of renewable sources of β cells, availability of biocompatible device and easily accessible site that facilitates minimally invasive clinical islet transplantation are to be addressed in this regard. Encapsulation was demonstrated to protect islets from host immune responses and thus has the potential to eliminate the need for immunosuppression. In addition, this approach might permit the use of allogenic and xenogenic islets that might obviate lack of renewable sources of β cells. Despite encouraging results obtained in animal models, loss of graft function in micro capsules (attributed to limited biocompatibility/ engraftment potential, immune rejection, hypoxia, and post-transplant inflammation) has limited their use in islet transplantation. The TheraCyte™ system provides with a device for encapsulating and transplanting cells is a membrane bound polymeric chamber. This chamber is fabricated from biocompatible membranes, which protect islet grafts from rejection by the recipient and also induce the development of blood capillaries close to the membranes, following subcutaneous transplantation. This offers a 3D scaffold as well as an additional immunoisolatory protection for transplant experiments. The present study used these immunoisolatory devices to test the viability and functionality of monkey islets in this devices following auto- or allo-genic transplantation in monkeys. The animal experiments were performed at National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad which has an approved primate animal facility. The research outcome will help us in understanding viability and functionality of monkey islets following transplantation using the TheraCytes™ devices and will also provide a basis to future transplantation of allogenic islet hormone-producing cells for replacement therapy in diabetic subjects.

AIMS AND OBJECTIVES

- Optimize protocols for islet isolation from monkey pancreas by following the gold standard "Ricordi" islet isolation procedure.
- To carry out auto or allogenic islet transplantation using TheraCytes™ implants at 2 different sites (Thigh and Neck region) in monkeys
- To test the viability and functions of isolated and transplanted islets before and during1 year of follow-up.

Work carried out

Islets were isolated by the standard method of using collagenase . They were tested for viability and functions ,and transplanted for a One yr follow up. All the parameters have been carried out before and after retrieval of the theracytes. The end points were monitored by biochemical (Body weight (kg), Blood glucose (mg/dl), cellular (IHC/SEM/TEM/FACS) and molecular (PCR) *in vitro*. The experiments include:

a) ***In vivo* glucose stimulated insulin release (GSIR).** *In vitro* GSIR was performed by challenging the islets with basal (5.5μM) and high concentration (16.5μM) glucose and estimating the insulin release into the medium. *In vivo* glucose stimulated insulin release was performed by I.V. infusion of glucose (0.5gm/kg) to the monkeys. Blood was collected from the femoral veins of both the legs and the blood collected from the non-theracyte region is considered as control. Insulin release in the blood was compared between the two thighs.

b) Insulin and Glucagon gene expression was carried out by IHC/ RT PCR.

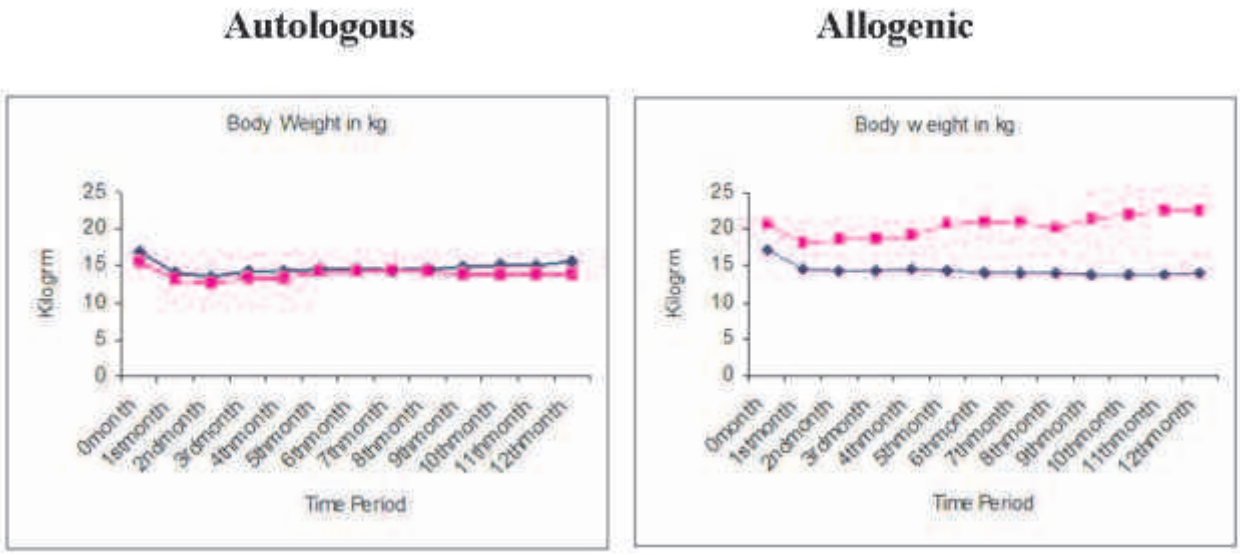
c) Electron micrographic studies for the presence of insulin secretory granules (TEM/SEM).

d) Calcium imaging studies (Using Calcium imaging specific software).

Results: Biochemical parameters

A) Body Weight (kg)

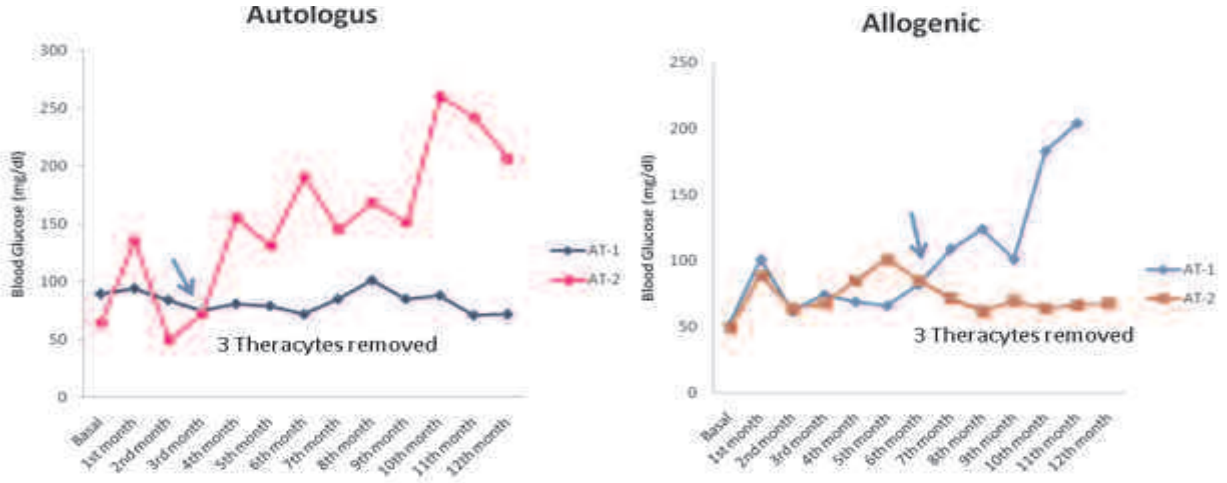
Partial pancreatectomy did not cause any changes in the animal body weight both in autologus and allogenic during 1 year follow up study.



Body weight of animals shows normal

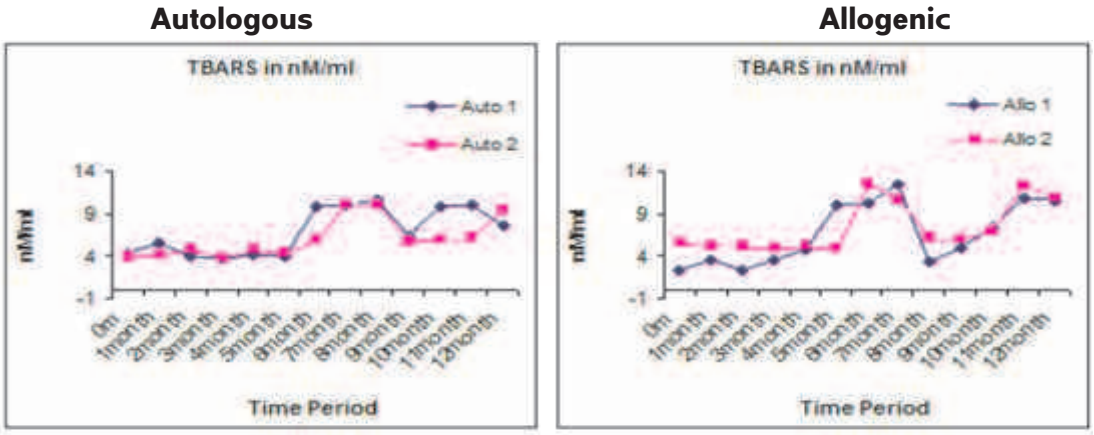
B) Blood Glucose (mg/dl)

Partial pancreatectomy resulted in marginal increase of blood glucose levels by the third postoperative day (from 70.8 ± 21 mg/dl to 109 ± 15 mg/dl), which returned to pre-transplant levels (71.6 ±12.6 mg/dL) by the second post-transplant week in all animals receiving either autologous or allogenic islet implants. Removal of three of the four islet loaded devices after 6 months and 10 months resulted in increased blood glucose levels (153 ±15mg/dl). Blood glucose levels BF and AF transplantation. The arrow indicates the time point when the animals had only one of the implanted theracyte left.



C) Global Oxidative Stress (TBARS)

Stress levels (TBARS levels) in serum showed significant increase after 6months retrieval as 3 theracytes from one animal and 2 theracytes from other animal from the each group was retrieved

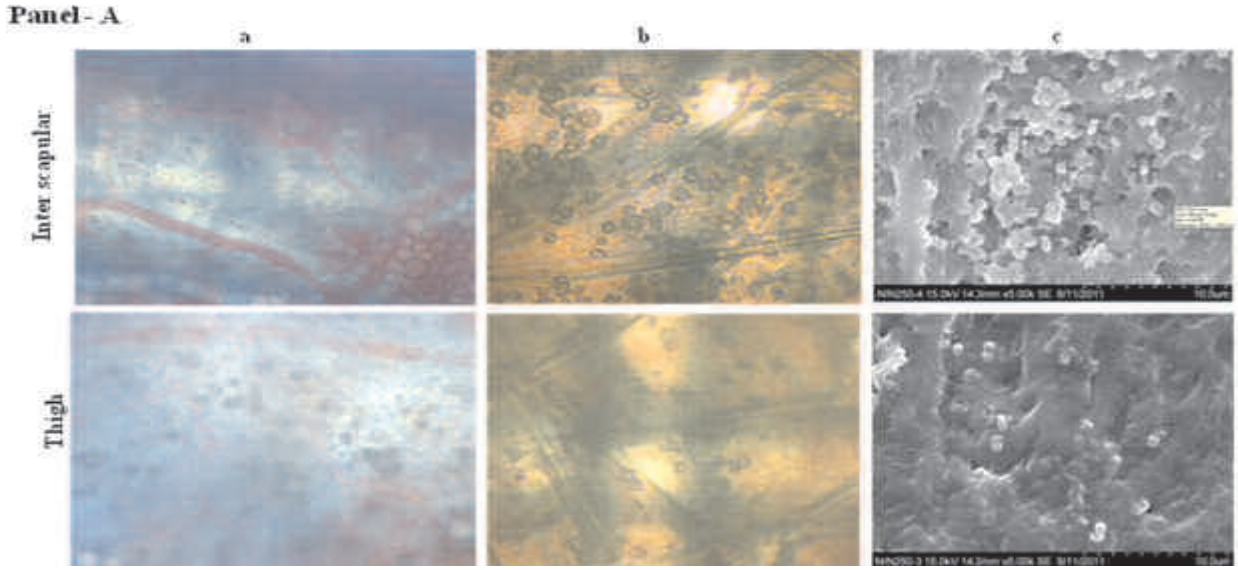


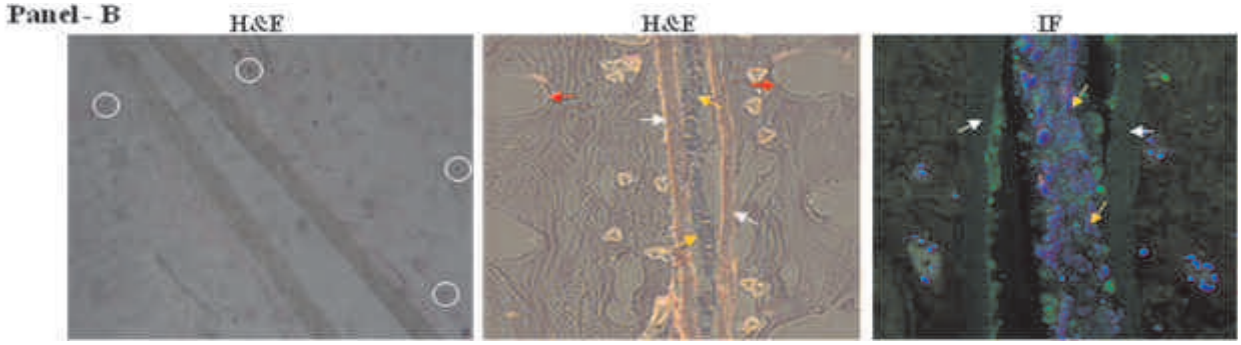
TBARS levels of animals that underwent auto or allo transplantation, Shows a rise after 6months.

Cellular parameters:

A) Vascularization and engraftment potential of the device

Islet-loaded devices, both from the thigh and interscapular regions at the end of 2, 6 and 12 months, revealed vascularization around the device which was deeply embedded in the subcutaneous tissues at the end of 12 months [Figure panel A (a)]. Light and scanning electron microscopy of the explanted device revealed the presence of islet clusters in them [Figure panel A (b),(c)]. It may be noted that vascularization and the number of islets within the device was more in devices implanted in the interscapular region, as compared to those implanted in the thigh, both in autologous or allogenic graft recipients (Figure panel A). On histopathological examination, granulation tissue was observed outside the device with occasional lymphocytes. Even though minimal fibrous tissue could be observed around the device at the end of 12 months, adequate number of vessels could be noted. While immune cell infiltration (CD4, CD8 cells and macrophages -CD68) was not evident, immunofluorescent staining of the explanted device confirmed the presence of insulin-secreting β cell mass and glucagon-secreting α cells (Figure panel B).

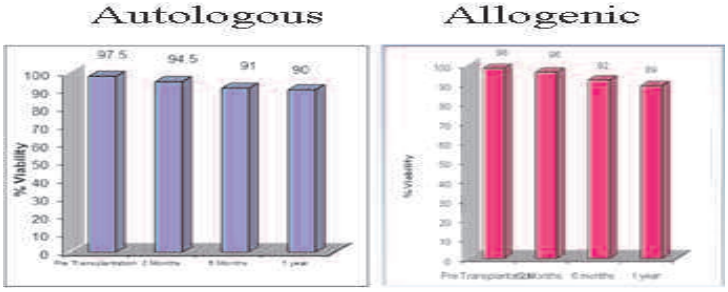




Panel-A: Assessment of retrieved theracytes from thigh and back of the animal as indicated (BackThigh). The retrieved theracyte showed vasculature (a). Normal morphology as indicated by Light microscopy (b), SEM (c) .Panel-B: H &E section and Immunofluorescence for Insulin.

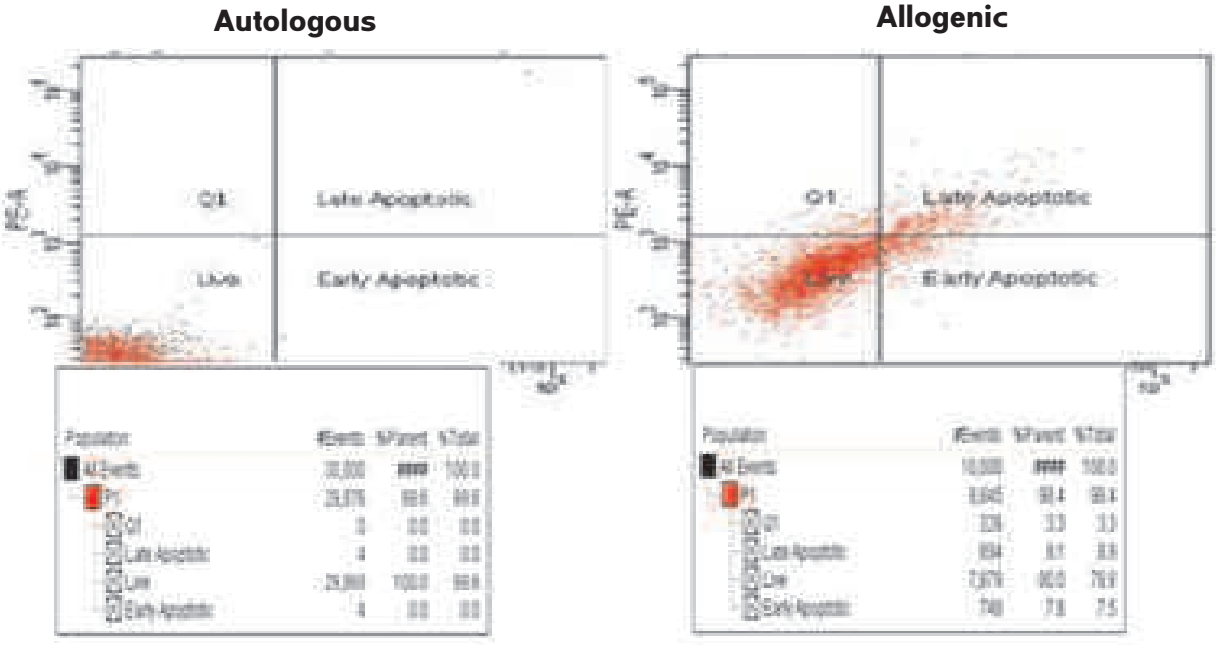
B). Viability of islets

Assessed by Trypan blue staining, Dithizone staining and MTT assay by the standard protocols. 90% in autologous and 80% in allogenic graft recipients with marginal decrease (≈ 10-20%) at the end of 12 months.



C) Viability of cell mass by FACS analysis

The beta cells were sorted on FACS based on autoflorescence from the retrieved theracytes and analysed by FACS and annexin V staining. These studies indicate that more than 80% of the sorted beta cells were viable in both auto- and allogenic islet transplanted animals without any immuno suppression treatment regimen.

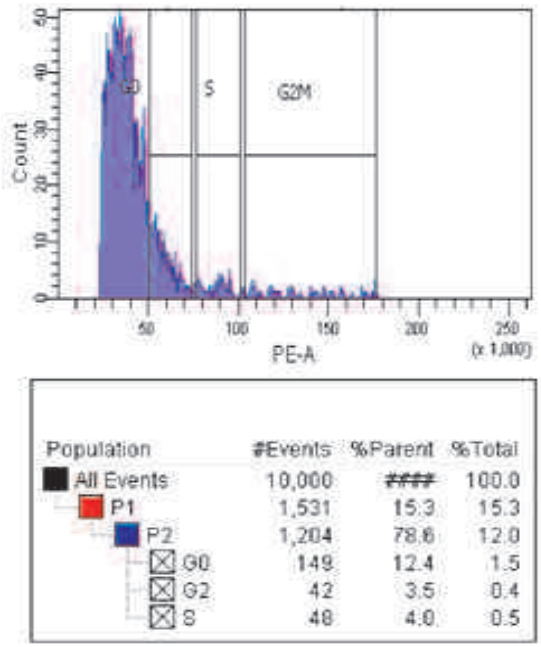


Assessment of the islet viability. The top panel shows the viability of the beta islet cells as assessed by trypan blue staining. The graph is a representation of one each for auto and allo transplantation. The lower panels show the annexinV staining followed by FACS analysis.

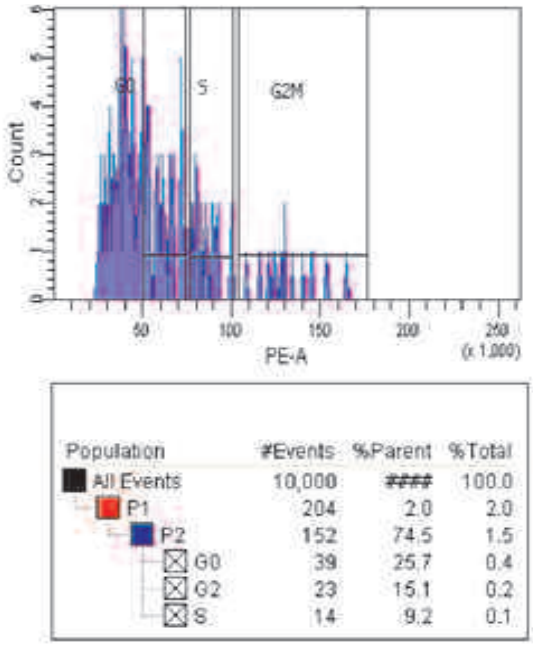
D) Cell Cycle analysis of the cells recovered from theracytes

Cell cycle analysis by flow cytometry indicated that greater number of recovered β cells were in active mitotic phase (24%±8) in comparison to those before implantation (7%±2) correlating with the increased number of islet cells in the device recovered at the end of 12 months.

Pre-transplantation



Post-transplantation

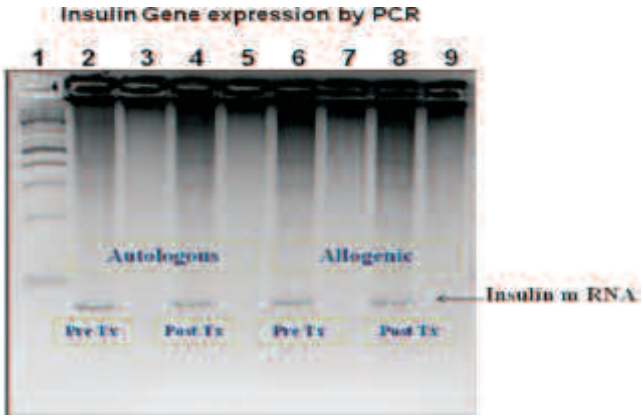


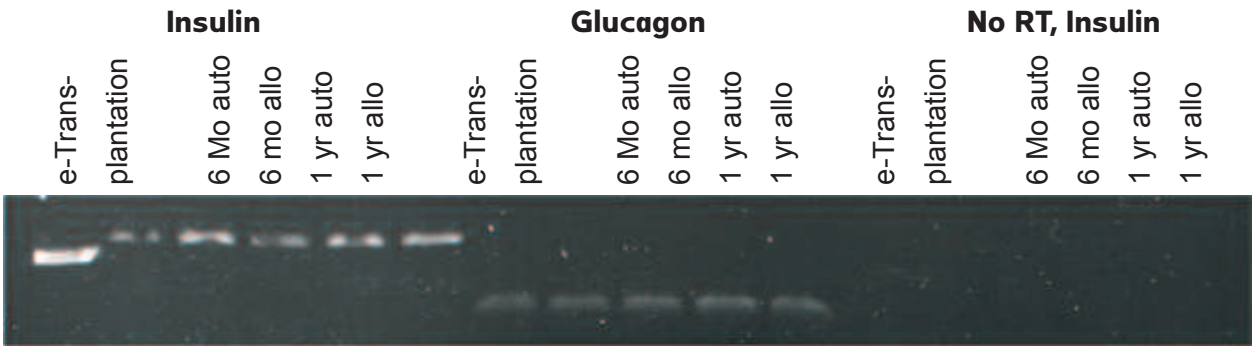
Cell cycle analysis of the beta cells isolated before and after transplantation

E. Molecular Characterisation

Expression analysis of insulin and Glucagon

Expression of mRNAs specific to insulin and glucagon were measured in freshly isolated islets before their implantation, or in islets recovered from the explanted devices. While the expression of genes coding for insulin and glucagon could be noted in such islets, concerned gene expressions were unaltered in islets recovered at the end of 12 months.



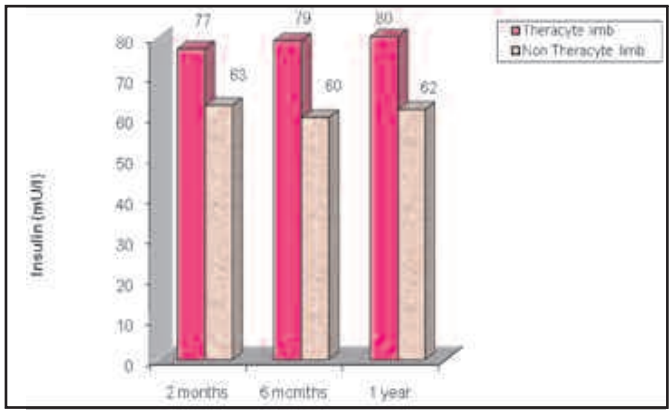


Insulin mRNA levels were assessed by quantitative RT-PCR.

RT-PCR reaction was carried out using RNA isolated from islet (pre and post transplantation) and insulin gene specific primers. The middle panel shows a similar representative gel for both glucagon and insulin expression from islets recovered after the auto and allo transplantation.

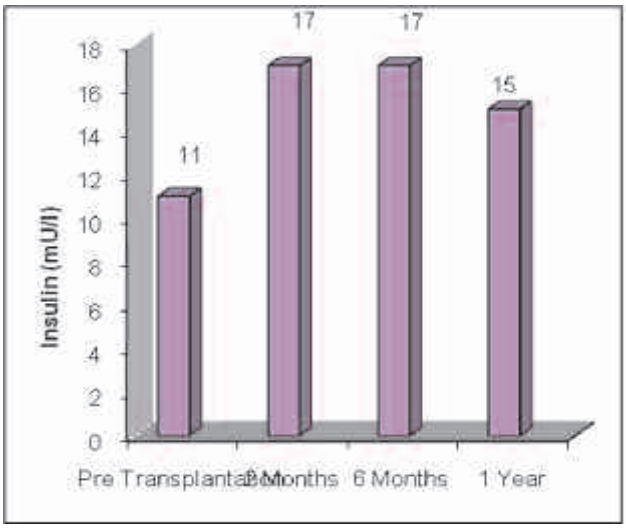
Functional assessment of islets after transplantation

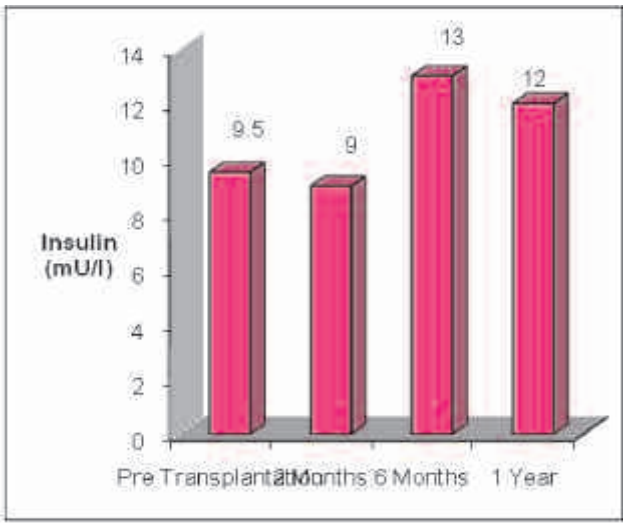
A) In vivo Glucose Challenge: *In vivo* assessment of islet functions after IVGTT (0.5 g/kg body) in the blood collected from femoral veins showed insulin secretion from the implanted islets. Insulin secretion was more (1.5-2.0 fold) in blood drawn from femoral vein of the thigh with the implanted device, as compared to the thigh without the implant, suggesting that the encapsulated islets respond to secretogogues.



In vivo glucose challenge. Glucose was injected IP and the insulin levels were assessed on both the thighs of the animal one that contained the theracyte and the other no implant.

B) In vitro Glucose Challenge: Challenging the explanted device containing islets with high glucose concentrations (16.5 mM) corroborated this observation indicating that the secreted insulin was indeed emanating from the device. Both in autologous and allogenic islet graft recipients, GSIR by islet-loaded devices, explanted at the end of 6 months of implantation, was noted to be 30% more when compared to those before.



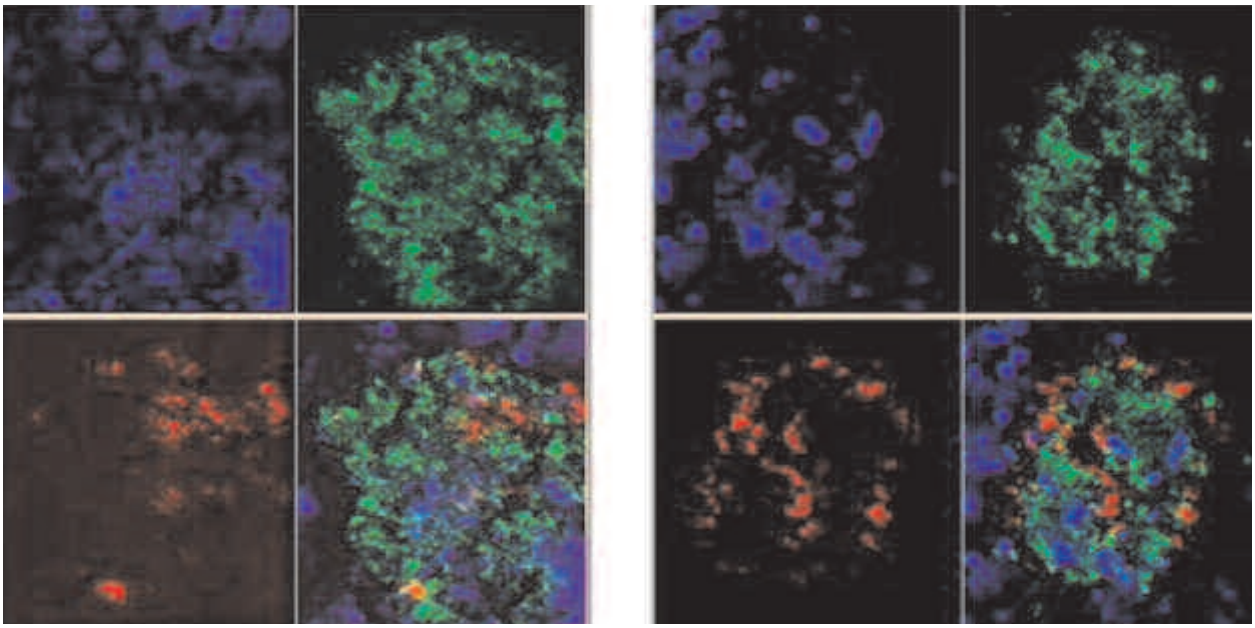


Glucose stimulated insulin levels were assessed for isolated theracytes by ELISA. The left panel shows the data for the theracyte recovered after autologus transplantation and the right panel shows the data for the theracyte recovered after the allo transplantation.

C) Retrieved Islets for Immuno-cytochemistry:

Islet (12 months)-Immunocytochemistry

Autologous **Allogenic**



Insulin producing cells **Glucogon producing cells**

8 ESTABLISHMENT OF PROPAGABLE CELL LINES FROM ADIPOSE TISSUE OF EMBRYONIC AND ADULT WNIN MUTANT OBESE RATS (WNIN/Ob AND WNIN/Gr-Ob)

Obesity with pre-clinical diabetes (hyperinsulinemia with impaired glucose tolerance/insulin resistance) is a multifactorial disorder affecting people worldwide and a potent risk factor for several micro and macrovascular complications, nephropathies, myopathies and cancers etc. Several animal models of obesity (ob/ob and db/db mice, Zucker rats) did help in understanding the pathophysiology and associated secondary complications of obesity/metabolic syndrome (MS), but extrapolating their observations to the human scenario is confounded by several factors. WNIN OBESE RATS (WNIN/Ob AND WNIN/GR/Ob) developed at our animal facility being an inbred model mimicking the classic X Syndrome (a combination of obesity and impaired glucose tolerance) scores an edge over other existing models in depicting the pathophysiological changes seen among human subjects.

Ex vivo systems such as cell free system, cell lines and primary cultures, facilitate in translating the *in vivo* events to an *in vitro* system allow for an in depth study of the characterization of cell physiology at biochemical and molecular level with no dependence on live animals and provides a continuous supply of biological material. As reported in the earlier annual reports (2008-2009, 2009-10, 2010-2011 and 2011-2012) have demonstrated for the insult of obesity (WNIN/Ob) and

obesity with impaired glucose tolerance (IGT) (WNIN/GR-Ob) at the level adipose tissue (subcutaneous and visceral) and the resident progenitor pool (mesenchymal stem cells in the stromal vascular fraction) *in vitro*. A similar insult of obesity with pre-clinical diabetes among Mutant rats was also seen from other multipotent adult progenitor sources such as bone marrow (gold standard for mesenchymal stem cells) derived mesenchymal stem cells confirm the promises of mesenchymal stem cells as an *ex vivo* tool to study the cellular, biochemical and molecular mechanisms underlying obesity and/or obesity with pre-clinical T2D.

AIMS AND OBJECTIVES

- To develop primary cultures and continuous cell lines from pancreas and adipose tissue from WNIN mutant obese rats
- To characterize the cell population for specific cell / tissue markers
- To cryopreserve the established cell lines for supply

METHODOLOGY

Anthropometric and Physiological Measurements: Anthropometric measures such as body weights and adipose depot weights (subcutaneous and visceral adipose depots) were assessed in WNIN Mutant rats (WNIN/Ob and WNIN/GR-Ob) at 35 days, 3months, 6months, 9months and 12months among Obese, Lean phenotypes along with their age matched parental controls (WNIN) and values are represented as Mean±SE.

Blood and plasma measurements such as blood glucose, plasma insulin, plasma triglyceride were performed from 2-3 ml of blood was collected by retro-orbital vein puncture from Mutant, Lean and Control rats at all ages as per standard protocols. Insulin resistance an important feature of obesity and pre-clinical T2D diabetes was computed mathematically from the fasting blood glucose (mg/dl) and fasting plasma insulin (µU/ml) using Homeostasis model of assessment (HOMA) from indices such as fasting glucose to insulin ratio (FGIR), Homeostasis model of assessment for insulin resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI).

Estimation of global oxidative stress levels by TBARS: Plasma thiobarbituric acid reactive species (TBARS) (measure of global oxidative stress) in plasma, subcutaneous and visceral adipose depots and the primary cultures of AD-MSCs from these depots by spectrophotometric measurement of the molnaldialdehyde (MDA) levels released by reaction with thiobarbituric acid (TBA) at 425nm.

***Isolation and Primary cultures of MSCs*:** Single cell suspension cultures of type II collagenase (Sigma) (initially 0.1% collagenase + 1% BSA and later 0.2% collagenase and 2% BSA) digested adipose tissues from subcutaneous (SCAT) and visceral (retro-peritoneal) (RPAT) fat pads were established from lean and obese phenotypes of WNIN/GR-Ob and WNIN/Ob of different age groups (35 days, 3months, 6 months, 9months and 12 months) in T25 flasks in DMEM/F12 medium (GIBCO) supplemented with 10-15% FBS (GIBCO) under optimal conditions (37^{O}C and 5% CO_2). At confluence, cells were passaged and characterized for morphology, Immunophenotyping, stress responses, multi-lineage differentiation and memory of the disease pathophysiology at the molecular level.

Population doubling assay: PDA was carried out at P3 as a function of time 24, 48, 72 and 96h in order to calculate the proliferation status where NH is the Harvested number and N1 is the plated number using the formula X = [log10(NH) - log10(N1)]. For the data presentation, values are given for the 35 days month age group from both Ob/Ob and GR-Ob animals.

***Immunohistochemistry*:** Immunostaining of the adipose tissue (for apoptosis by TUNEL/ Caspase-3) and adipose derived mesenchymal stem cells (Mesenchymal marker Stro-1, TNFa-PE) was performed as per the published protocol.

***FACS analysis for surface marker profile of AD-MSCs*:** Mesenchymal specific surface marker staining for CD90-PE, CD29-FITC, CD31-PE was performed as per the reported protocol and analyzed in FACS Aria II (BD, San Jose, CA) using FACSDiva software (BD, San Jose, CA) among AD-MSCs from Obese, Lean and Control at all the age groups defined. A total of 10,000 events were acquired to determine the positivity of different cell surface markers used.

***Gene expression studies by RT-PCR*:** cDNA prepared from 4? g of isolated total RNA (using Trizol Reagent (Invitrogen)) from adipose tissues and primary cultured cells among all the phenotypes and age groups was amplified using gene specific primers to study the changes in the gene expression levels among different phenotypes with age in obesity/pre-clinical T2D at both the tissue and cellular levels.

***Differentiation potential*:** Functional characterization of the cultured AD-MSCs (SCAT and VAT) from all the phenotypes and with age were performed by differentiating these MSCs using standard inducing agents (Chemicon, USA) to give rise to lipid droplet-filled cells (adipocytes) at the end of the differentiation protocol (15 days), which stain positive with Oil red.

Karyotyping: To study the genetic stability of the cultured cells through different passages, the cultured cells were karyotyped at CCMB using the G-banding (Giemsa Staining) by standardized protocols.

RESULTS

A. Physiological Measurements

Anthropometric measurements

- Increased body weights among Mutants (both WNIN/Ob and WNIN/GR-Ob) compared to Lean and Control with age (12m≈9m>6m>3m>35 days).
- Corresponding increase in depot weights (SCAT and RPAT) with age also observed (among both WNIN/Ob and WNIN/GR-Ob).
- Body and adipose depot weights among WNIN/Ob and WNIN/GR-Ob were comparable.

Blood and Plasma Measurements

- Both WNIN/Ob and WNIN/GR-Ob were normoglycemic (50-110 mg/dl) at all ages studied (among Obese, Lean and Control).
- Obese from both WNIN/Ob and WNIN/GR-Ob demonstrated hyperinsulinemia.
- Insulin resistance (from HOMA-IR, FGIR, QUICKI) was demonstrated even at 35 days among WNIN/GR-Ob Mutants, and from 6 months among WNIN/Ob rats.
- Hypertriglyceridemia was also demonstrated among Obese (Obese>Control≥Lean) among both WNIN/Ob and WNIN/GR-Ob, which also showed an increase with age (12m>9m>6m> 3m> 35days).

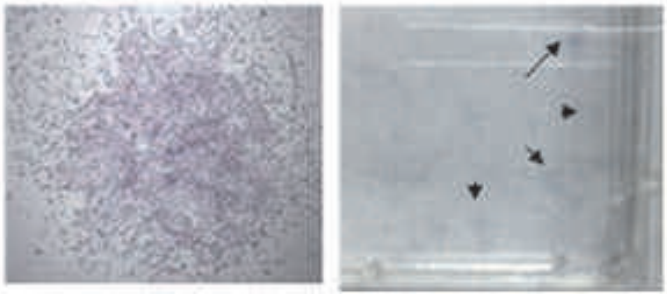
B. Studies on adipose-derived mesenchymal stem cells

Isolation and Characterization of Primary cultures of MSCS

Colony-formation unit (CFU) assay:

- Primary cultures of SCAT showed rapid adherence to plastic and adherent cells demonstrated spindle cell morphology.
- Mesenchymal nature was also reflected by the Colony-formation units demonstrated among both WNIN/GR-Ob and WNIN/Ob Mutant rats

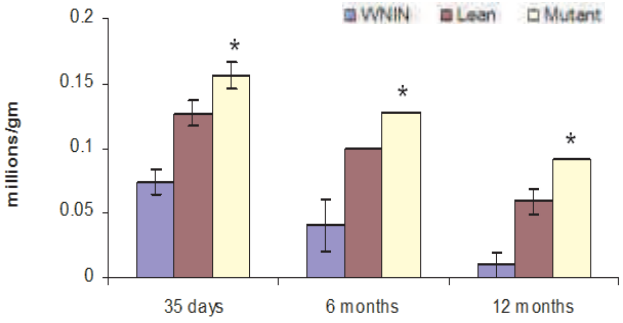
Hematoxylin stained colony (colony forming unit) from AD-MSCs (10X) magnification



Arrows indicate location of multiple CFUs in the culture flask

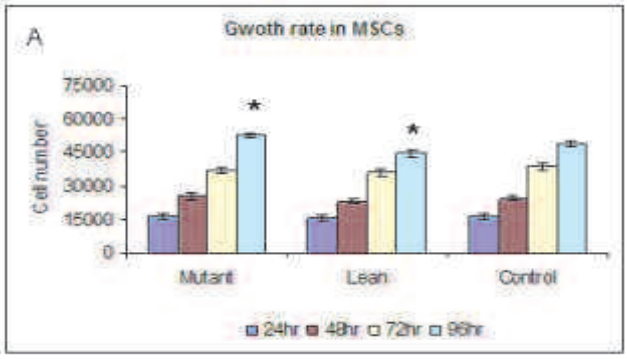
Yield of MSCs:

- Yield of AD-MSCs (SCAT and RPAT) (SVF) was found decrease with increasing age (35d>3m>6m>9m>12m).
- Yield of SVF greater in visceral depots (RPAT> SCAT).
- Yield of AD-MSCs similar among WNIN/Ob and WNIN/GR-Ob.



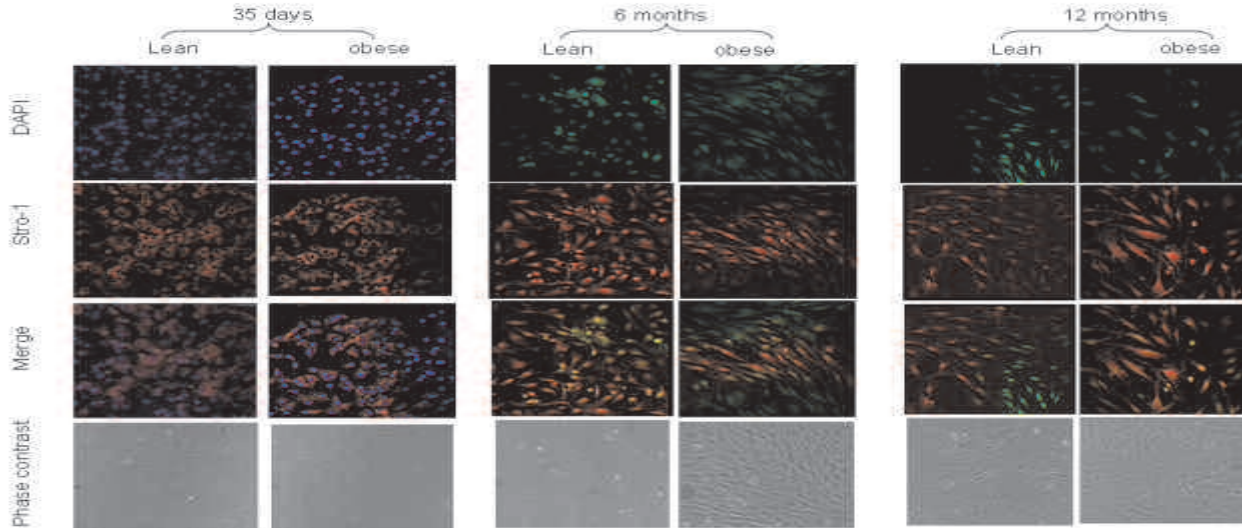
Proliferation rates

- Proliferation rate was maximum at 35 days in WNIN/GR-Ob mutants confirmed by PDA assay.
- Increased 'S' phase of the cell cycle among AD-MSCs from Mutants was observed at 35 days from both Ob/Ob and GR-Ob (35d>3m>6m>9m≈ 12m) age observed among AD-MSCs from Mutants analyzed by cell cycle analysis using propidium iodide staining in FACS.



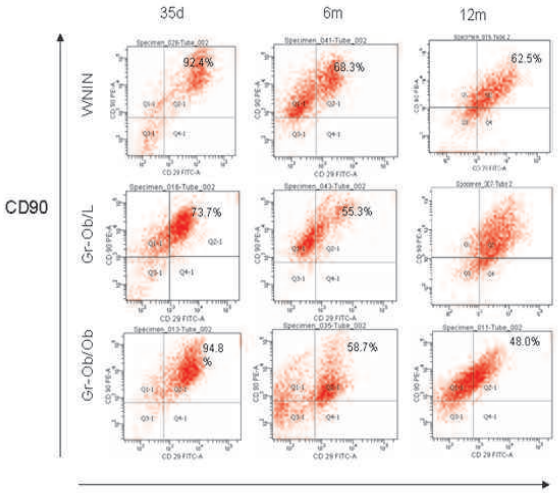
Characterization of AD-MSCs

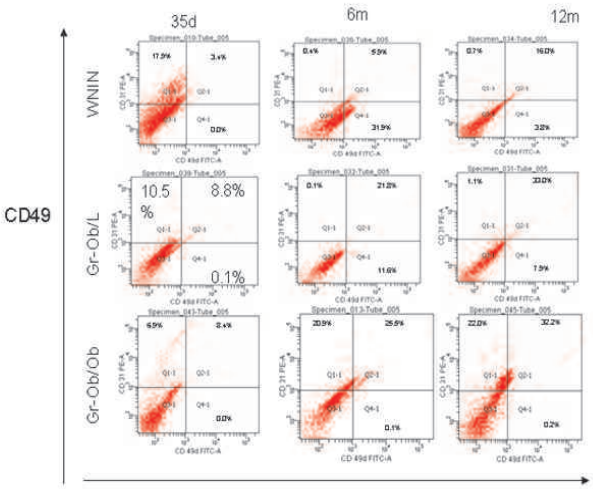
Primary cultures of AD-MSCs were predominantly STRO-1 positive resembling that of a mesenchymal population.



Staining of these MSCs for mesenchymal stem cell specific positive (CD29, CD90) and negative surface proteins (CD31) and evaluation by FACS further confirmed their mesenchymal nature.

- Increased percentages of $CD90^{+}CD29^{+}$ (double positive) populations among cultured AD-MSCs from obese (both WNIN/Ob and WNIN/GR-Ob). However, among the Mutants $CD90^{+}CD29^{+}$ in WNIN/GR-Ob > WNIN/Ob.
- Percentages of $CD90^{+}CD29^{+}$ populations decreased with age (35d>3m>6m>9m>12m).
- Cd31 used as a negative marker was expressed at low levels among both WNIN/GR-Ob and WNIN/Ob phenotypes at all the age groups studied.



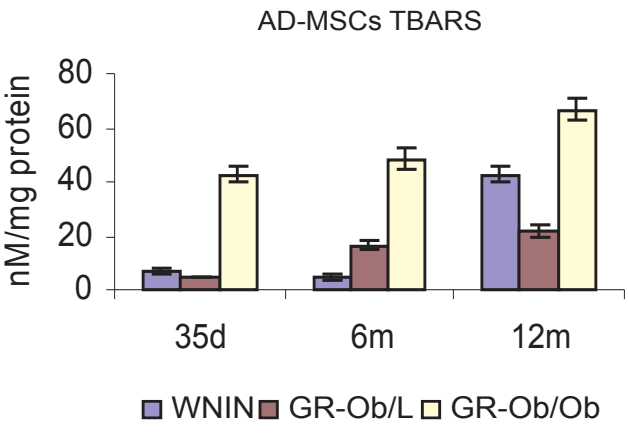


CD90/CD29 %+ve	35d	6m	12m
WNIN	92.4	62.5	45.7
Gr/L	73.7	55.3	51.2
Gr/Ob	94.8	58.7	48

CD31 % -ve	35d	6m	12m
WNIN	0.1	0.9	1.1
Gr/L	0.1	1.1	1.3
Gr/Ob	0.2	2.7	0.6

Stress level measurements

- Levels of plasma TBARS were found to be consistently higher in obese phenotype compared to both lean and WNIN and increased with age i.e. at 6 months.
- A similar increase in TBARS was also observed among AD-MSCs from Mutants (WNIN/Ob and WNIN/GR-Ob) compared to Lean and Controls (Mutants> Lean> Controls) and also with age (12m>6m> 35d). Interestingly, higher TBARS levels were observed among VAT-MSCs (VAT>SCAT).



Gene expression studies by RT-PCR

A summary of changes in gene expression by densitometry with age and among the phenotypes Mutant, Lean and Control (among both WNIN/Ob and WNIN/GR-Ob) represents in the table below;

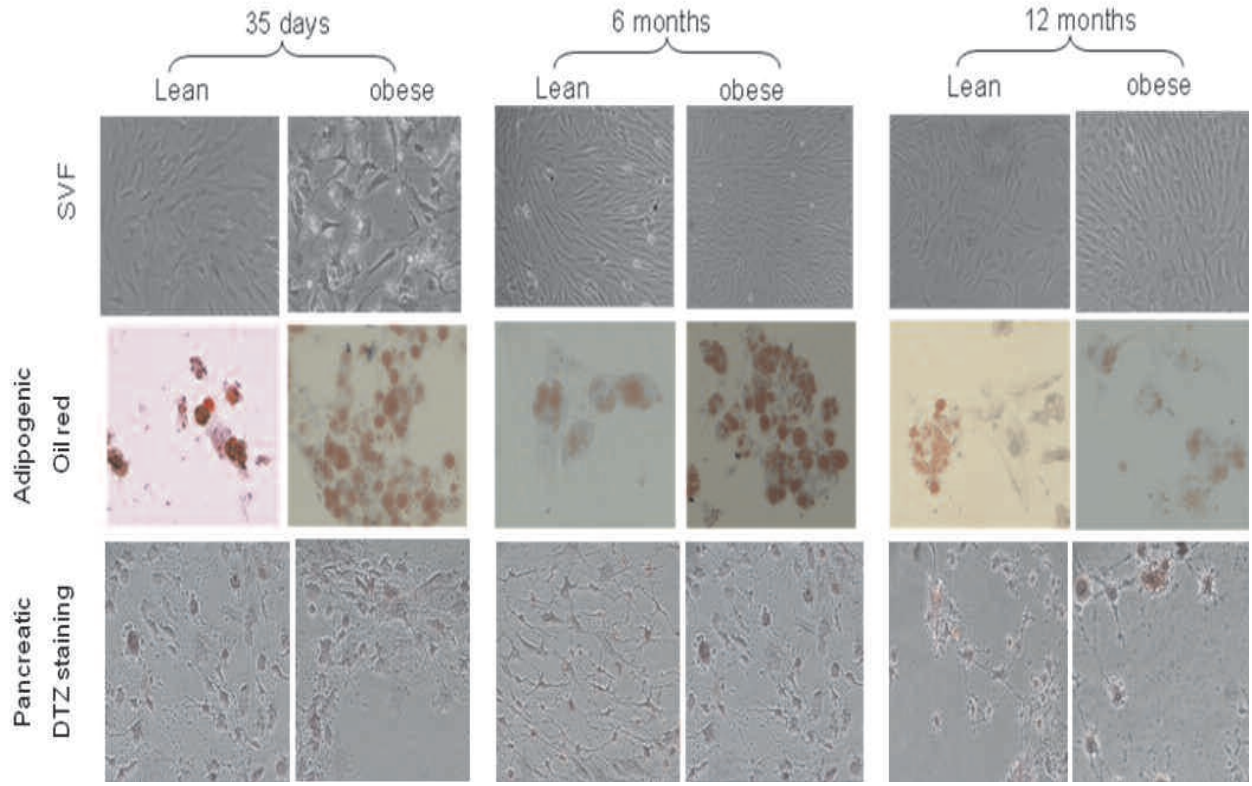
Table Summary of gene expression profile among Stromal Vascular Fraction

Group	Markers	SVF	
		Peak	age
Embryonic markers	Oct-4	35 days	↓
	Sox-2	35 days	↓
	Nanog	12 months	↑
Mesenchymal markers	Dact-1	35 days	↓
	Pref-1	35 days	↓ (6m) ↑
Adipogenic transcription factors	PPARg2	35 days	↓
	SREBP-1C	35 days	↓
	C/EBPa	35 days	↓
Mature adipocyte markers	Leptin	6 months	↑ (6m) ↓
	Adiponectin	35 days	↓
	LPL	6 months	↑ (6m) ↓
	aP2	35 days	↓
	GLUT-4	6 months	↑ (6m) ↓
	IRS-1	35 days	
Inflammatory markers	IL-6	35 days	↓
	TNFa	35 days	↓
Senescence Markers	Telomerase	12 months	↑

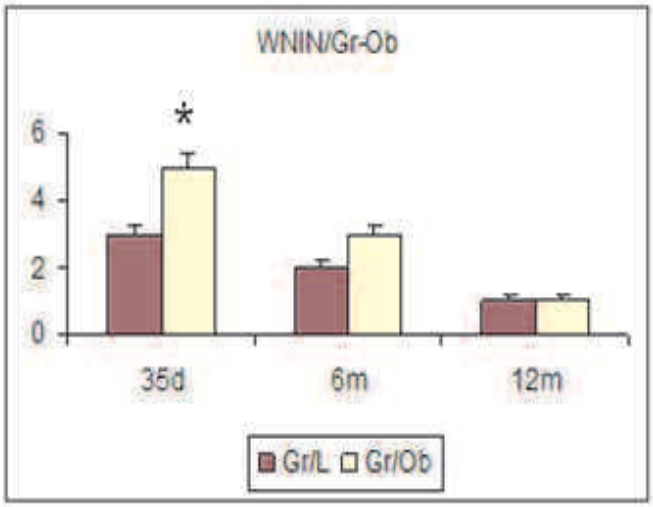
The data shows an upregulated expression of the following markers among GR-Ob and Ob/Ob phenotypes,

- Progenitor (embryonic and mesenchymal) specific markers – Oct-4, Sox-2, Dact-1 and Pref-1 (35d>3m>6m>9m>12m)
- Adipocyte transcription factors PPAR 2, SREBP-1C and C/EBP? ; mature adipocyte specific – adiponectin and lipid transport related aP2, (35d>3m>6m>9m≈12m)
- Inflammatory cytokines – IL-6 and TNF (35d<3m< 6m <9m<12m).
- Mature adipocyte markers leptin, GLUT-4 and LPL reached a peak by 6 months, as compared to 35 days, and showed a slight decrease by 12 months.
- These observations depict a state of oxidative/inflammatory stress underlying obesity (WNIN/Ob) and obesity-induced diabetes (WNIN/GR-Ob).

Differentiation Potential

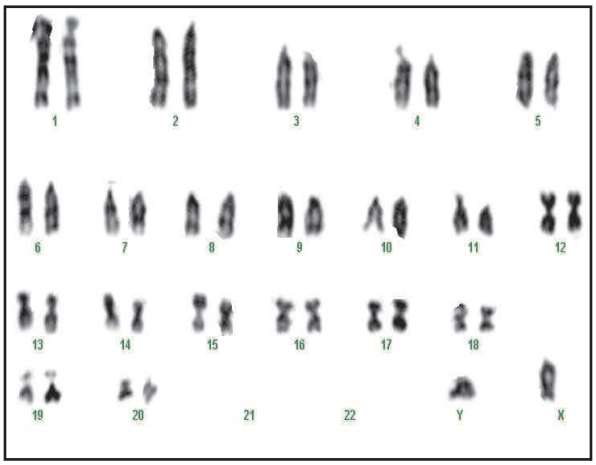


- Higher adipogenic induction was among Obese (SCAT and RPAT) and with age (35d> 3m> 6m> 9m> 12m) from both WNIN/Ob and WNIN/GR-Ob compared to their Leans and Controls. Also the content of triglycerides in these differentiated adipocytes (oil red staining) was found to decrease with age (35d> 3m> 6m~ 9m> 12m).
- This was also evident from the increased gene expression levels of key adipogenesis related genes – Leptin, LPL, aP2, GLUT-4, PPAR 2 among SCAT from 35 days mutants (WNIN/Ob and WNIN/ GR-Ob).

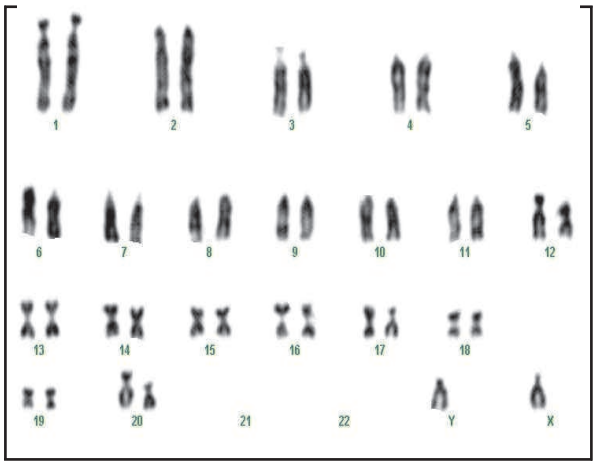


- Since these Mutant rats demonstrate traits of insulin resistance/impaired glucose tolerance we also demonstrated the transdifferentiation potential of AD-MSCs to insulin-secreting-like cells/clusters as indicated by DTZ staining (specifically binds to Zn molecules in insulin to give deep crimson red colour).
- Transdifferentiation potential of the AD-MSCs was similar to the observations from adipogenic lineage commitment being higher among Obese (Obese>Lean>Control). A similar decrease in the transdifferentiation potential was also observed with age (35d>6m>12m).

Karyotyping



35d Mutant SCAT P8



6m Mutant SCAT P8

- MSCs derived from both the Mutant rats (WNIN/GR-Ob and WNIN/Ob) demonstrated an normal karyotype at both lower (P2) and higher passages (P10)

Highlights of the work on AD-MSCS

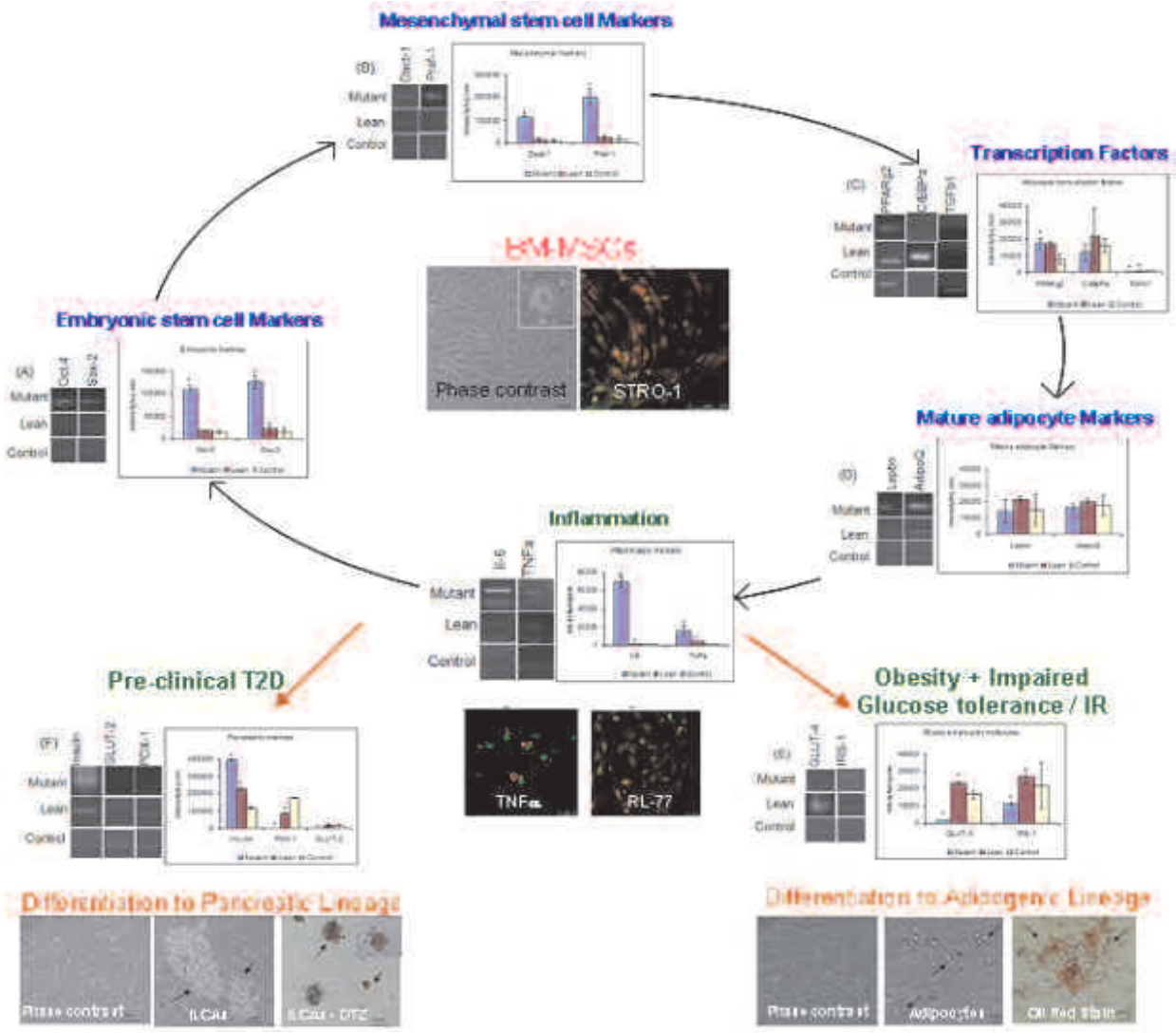
About 35 days WNIN/GR-Ob and WNIN/Ob mutant rats can be advocated as a promising model to study Obesity /T2D as compared to Lean and Controls: The features are,

- Increased yield of MSCs (SCAT),
- Increased proliferate rate studied by PDA and cell cycle analysis (SCAT),
- Upregulated expression of stem cells, preadipocyte, and adipocyte differentiation markers
- Karyotype of the cultured cells at 35 days and 6 months is normal (P3 & 8)
- Adipogenesis to form adipocytes was highest in Ob/GR-Ob mutants compared to their Lean and Controls.

C. Studies on Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs)

To confirm features of increased stem cell recruitment to the adipogenic lineage, increased levels of cellular, oxidative and inflammatory stress levels seen among the adipose tissues and their resident progenitors (AD-MSCs from SCAT and VAT) of WNIN/GR-Ob Mutant rats, thus probably contributing to the vicious cycle of increased adipogenesis and adipose depot sizes as seen in Obesity, we studied if these features were reflected in the bone marrow derived mesenchymal stem cells (BM-MSCs) (considered as the gold standard for multipotent adult mesenchymal progenitor population). Observations from this part of the study (summary of these observations has been represented in the figure below) do demonstrate the insult of obesity/obesity with pre-clinical diabetes seen in situ among WNIN Mutant obese rats at the level of bone marrow which is known to the harbor of pluripotent stem cells in addition to multipotent stem cells and hence is involved in progenitor recruitment to various tissues in the body and has been published by us recently.

Based on findings from both AD-MSCs and tissue characterization we have propose the Hypothetical model for changes in the gene expression patterns in obesity /T2D similar to human situation.



D. Tissue characterization

To substantiate our observations on the cultured SVF fraction, we also studied the subcutaneous and visceral adipose tissues of WNIN/Ob and WNIN/GR-Ob rats at all the age groups. The results of these studies are summarized below.

Adipocyte Size

- The adipocyte size was found to increase with age 35<6>9months in the WNIN/Ob and WNIN/GR-Ob phenotype showed consistent increase in the adipocyte size from the age 35<6 <9 months.
- Adipocyte size was found to be larger among RPAT compared to SCAT in line with several previous reports on adipocyte size.

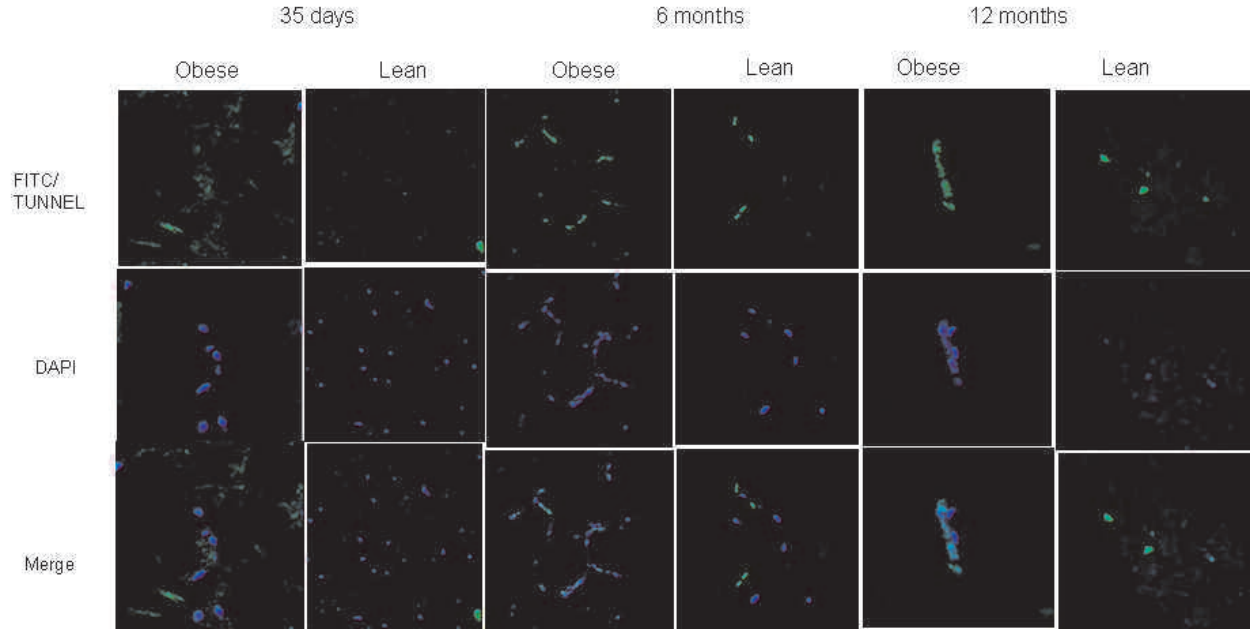
Stress levels

TBARS

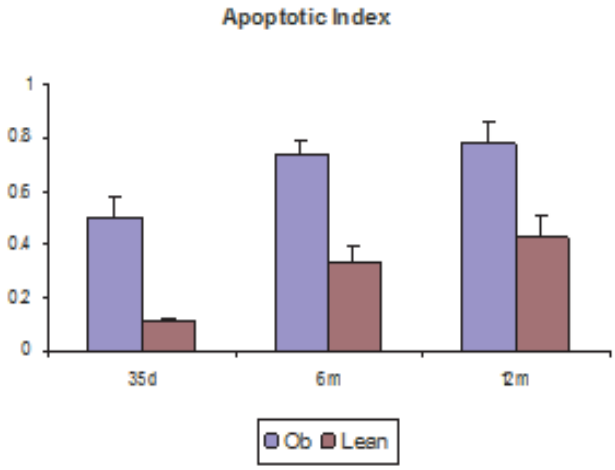
- A similar in TBARS levels were also observed at 6 months in SCAT and RPAT tissue depots of Ob-Ob phenotype as compared to WNIN/GR-Ob.

Apoptosis measurements

- Adipose tissues from WNIN/Ob /GR-Ob mutants demonstrated decreased expression of adiponectin, GLUT-4 12>6>35> months.
- ER stress 12>6>35> months in par with other markers (TBARS and apoptotic rates).
- Apoptotic index showed an increase 12>6>35> months.



Gene expression analysis: Upregulated expression of Embryonic markers such as Sox-2, Preadipogenic markers (Pref-1),and Adipogenic transcription factors (PPAR? 2) and mature adipocyte markers (Leptin, LPL, aP2) among WNIN/GR-Ob and WNIN/Ob mutant rats as compared to their lean and Controls.



Highlights of the work on Adipose tissues

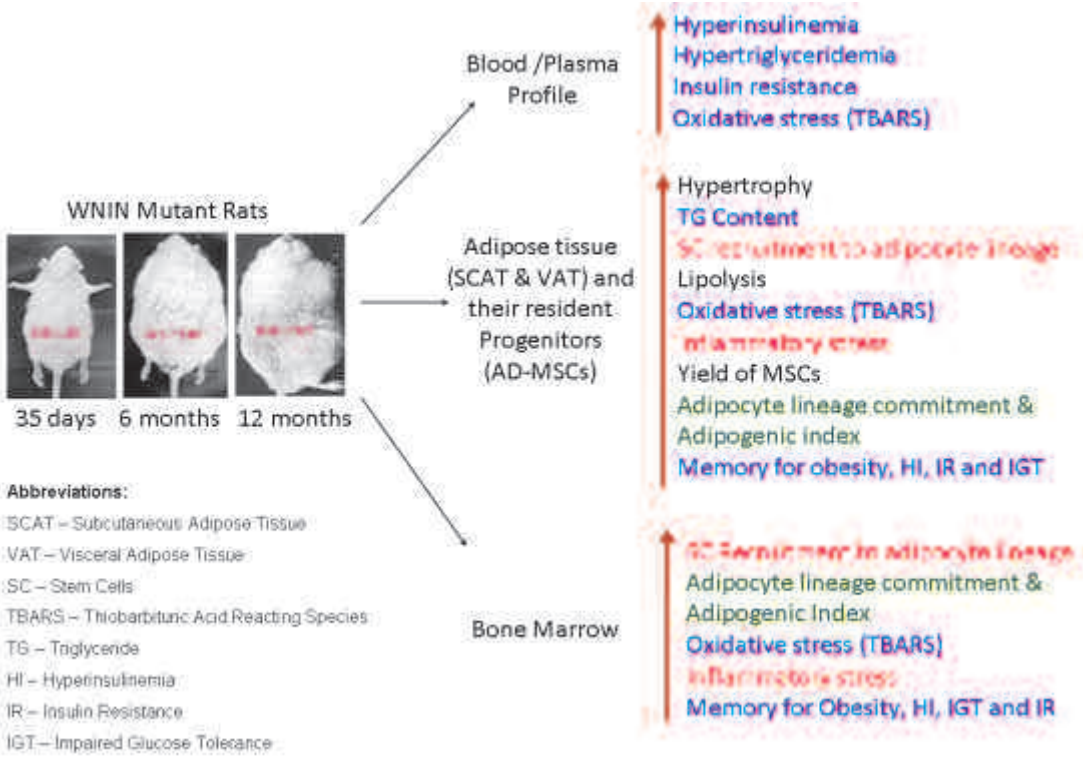
WNIN Mutants rats (WNIN/GR-Ob and WNIN/Ob) demonstrated a state of profound stress evidenced by,

- Adipocyte hypertrophy
- Hyperinsulinemia with insulin resistance
- Upregulated cellular (TBARS and ER stress) and inflammatory stress (IL-6, TNFa) at all the three hierarchies (plasma, adipose depots and AD-MSCs from these depots),
- Increased adipogenic commitment seen at the tissue levels (Sox-2, Pref-1, PPAR? 2, LPL, IRS-1, GLUT-4) and an increased adipogenic potential at the level of SVF from the Mutant adipose depots (increased oil red staining on adipogenic induction).
- Features of accelerated aging seen at the levels of both SCAT and VAT and their SVF fractions (telomerase mRNA levels).

These Observations do unequivocally demonstrate for the memory of obesity with insulin resistance seen among obese and obese diabetic subjects at the levels of progenitors. Based on our findings from both AD-MSCs and tissue characterization we have propose the Hypothetical model (Figure) for changes in the gene expression patterns in obesity/ T2D similar to human situation.

Salient findings and Summary

Overall, the AD-MSCs from the WNIN/GR-Ob model system demonstrate features of obesity with IGT, IR and hyperinsulinemia similar to the observations *in situ* and the observations in human subjects with preclinical/clinical features of T2D.



9 CHARACTERIZATION OF ACTIVE PRINCIPLES AND MECHANISM OF ACTION OF DIETARY ALDOSE REDUCTASE INHIBITORS AND ANTIGLYCATING AGENTS: (V) ISOLATION AND CHARACTERIZATION OF -GLUCOGALLIN AS A NOVEL ALDOSE REDUCTASE INHIBITOR FROM *EMBLICA OFFICINALIS*

Diabetes mellitus is recognized as a leading cause of new cases of blindness throughout the world, and the rapid increase in the incidence of diabetes in recent years suggests that diabetic eye disease could become an even larger public health problem in the near future. Diabetic patients face a 25-fold increased risk of blindness as a result of diabetic retinopathy and/or cataract in comparison with the general population. While strict long term control of blood glucose can reduce the likelihood of developing retinal lesions leading to retinopathy, present methods for achieving strict metabolic control are not suitable for most diabetic patients because of excessive cost and complexity. Therefore, patient education, lifestyle modifications, and new technologies such as blood glucose monitors and insulin pumps collectively will still fall short of effectively preventing diabetic eye disease for the general population. Numerous clinical trials and experimental animal studies have shown that early intervention is required to achieve maximal reduction in the onset and severity of diabetic retinopathy and cataracts. Therefore, medical therapies developed to delay the onset and progression of diabetic eye disease must be sufficiently safe and well tolerated to allow lifelong treatment.

Many theories have been advanced to explain the pathogenesis of diabetic eye disease. These include excess formation of advanced glycation end-products (AGEs), activation of the glucosamine pathway, activation of PKC isoforms, and activation of the polyol pathway. The first step of the polyol pathway is catalyzed by aldose reductase (ALR2), which converts glucose to sorbitol with concomitant oxidation of NADPH to NADP. Accelerated flux of glucose through the polyol pathway has been implicated in the pathogenesis of diabetic eye disease. Several groups have reported that ALR2 becomes activated in diabetic tissues. Elevated ALR2 activity measured in erythrocytes was associated with risk for developing retinopathy among patients with type 2 diabetes were showed.

Given the close association between ALR2-mediated sorbitol accumulation and diabetic eye disease, considerable effort has been focused on developing ALR2 inhibitors to prevent diabetic complications. Previously we have shown that crude aqueous extracts from *Emblica officinalis* (Amla) fruit delayed the onset and progression of cataracts and normalized diabetes-induced markers of lipid peroxidation and protein carbonyls. Moreover, these studies demonstrated that the active component(s) of the aqueous extract penetrate the lens and substantially delay the progression of cataracts through ALR2 inhibition. In this study we present the isolation and structure elucidation of the naturally occurring ALR2 inhibitor from *E. officinalis* fruit to be 1-O-galloyl-b-D-glucose (β-glucogallin). Additionally, we show that β-glucogallin inhibition of ALR2 is specific over other aldo-keto reductases (AKRs) and active in an *ex vivo* transgenic lens organ culture, preventing the accumulation of sorbitol under hyperglycemic conditions.

METHODOLOGY

Isolation and structure elucidation of -glucogallin: Pericarp of *E.officinalis* was freeze-dried, powdered and extracted with water and the aqueous extract was lyophilized. The lyophilized aqueous extract was further extracted with 9:1 (acetone:water) solution and subjected to Sephadex® LH-20 flash chromatography. About forty-five of the 150 column chromatography

fractions showed significant activity against ALR2 *in vitro*. Material from the active fractions was further purified using bioassay-guided fractionation via C18 reversed phase HPLC, which revealed the highest inhibitory activity to be localized in one major fraction. The active pure fraction was subjected to ^{1}H and ^{13}C NMR as well as LC-MS for structural determination. Following structural elucidation, solutions of pure β-glucogallin in water were prepared for *in-vitro* and *ex-vivo* analysis.

Inhibition of ALR2 and other members of the AKR family: Pure β-glucogallin was tested for inhibitory activity against ALR2, AKR1B10 and AKRA1 as described previously by us.

Ex-vivo lens organ culture studies: To confirm our *in vitro* data, a transgenic mouse model, which overexpresses human ALR2 specifically in the lens was utilized. Transgenic mice designed for lens-specific expression of ALR2 were produced by standard methods on a C57BL6 strain background. Eyes were collected and lenses dissected into Dulbecco's Modified Eagle's Medium (DMEM) supplemented with insulin/transferring/selenium buffer, penicillin-streptomycin and fungizone, and were incubated in the presence of 95% O_2, 5% CO_2 at 37 °C. Lenses that remained clear after 24 h incubation were then switched to either DMEM containing 5.5 mM glucose (basal glucose) or DMEM containing 27.5 mM glucose (high glucose) in the presence or absence of glucogallin. At the end of the experiment, sorbitol content in the lenses was determined.

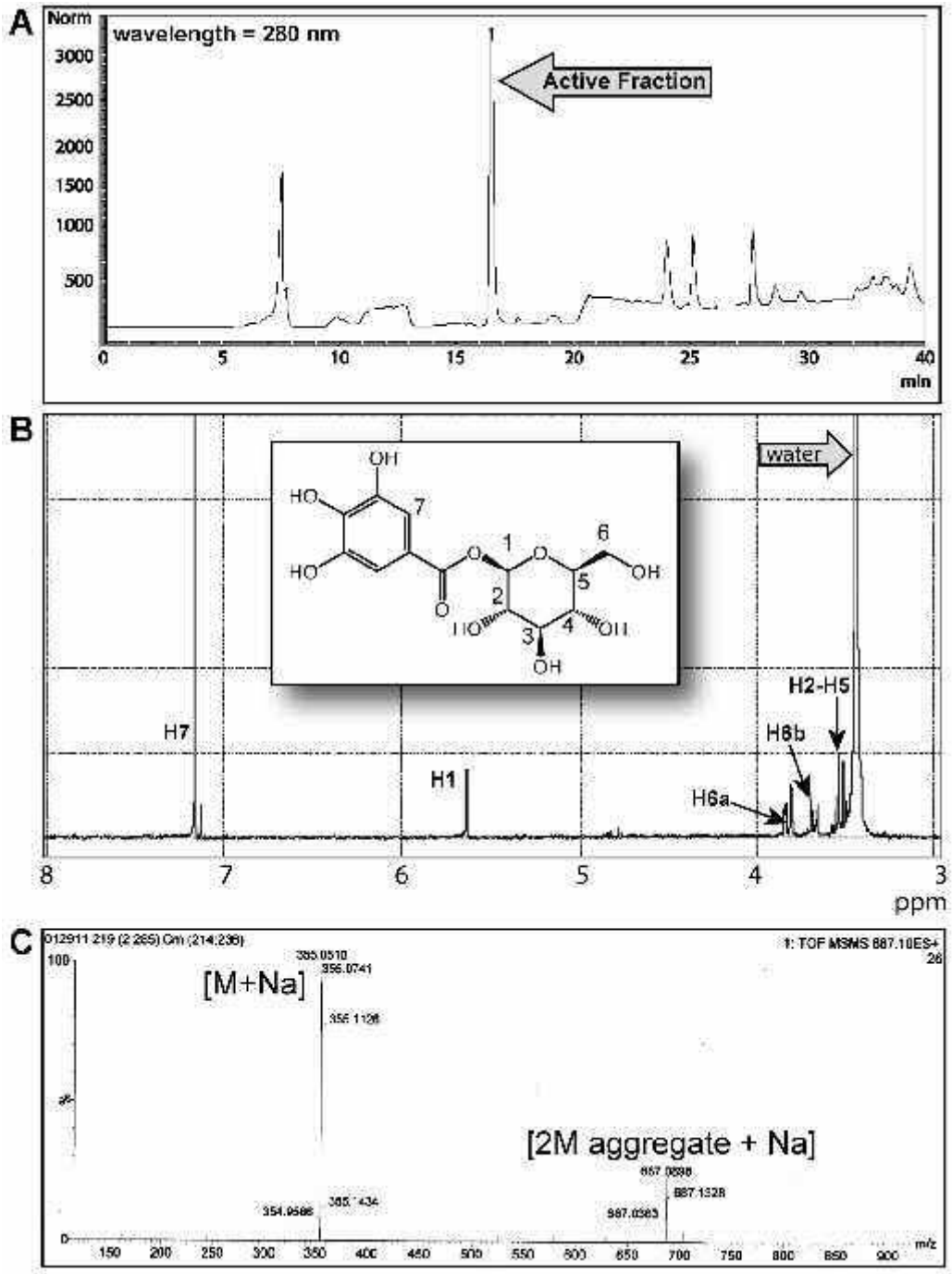
Computational modeling of -glucogallin with ALR2: Molecular docking was done by discovery (discover 2.7) package on an O2 (R12000) workstation. All ligand were minimized and least energy conformations were taken for docking studies. Crystal structure of ALR2 (or AKR1B1) was downloaded from Brookhaven data bank (PDB: 1PWM) and protein structure minimized by using charmM force field. All water molecules were removed. LigandFit was used for the molecular docking of β-glucogallin and sorbinil into the defined binding site of ALR2. The Poisson-Boltzmann method was used to calculate binding energy, with a non-bonded list radius of 12Å. Active site interactions were determined utilizing the Structure Monitor in Discovery Studio in conjunction with the create pharmacophore protocol in LigandScout.

RESULTS

1. Bioassays-guided fractionation of active fractions of LH-20 chromatography via C18 reversed phase HPLC revealed the highest inhibitory activity to be localized in one major fraction (Figure 1A).
2. Structural analysis (^{1}H and ^{13}C NMR, LC-MS, MSMS) confirmed the absolute structure as β-glucogallin. Moreover, there were no stray peaks in any of the analytical spectra, confirming that the only compound present in the purified active fraction was β-glucogallin (Figures 1B and 1C).
3. The IC_{50} value of β-glucogallin for ALR2 was determined to be 58 ± 3 M. In contrast, our results indicate a high degree of selectivity of β-glucogallin for ALR2 over the most abundant other members of the human AKR family, as negligible inhibition was observed when assays were conducted using AKR1B10 and AKR1A1 (Figure 2A).
4. Inhibition appeared noncompetitive in the Lineweaver-Burk plot (Figure 2B) as the slopes of the linear fits are increased for the solutions with β-glucogallin present and the y-intercept increases with increasing concentrations.
5. β-glucogallin (30 μM) can penetrate lens tissues *ex-vivo* and successfully inhibit ALR2 under hyperglycemic conditions, preventing the accumulation of sorbitol and subsequent osmotic stress (Figure 3).
6. The phenolic moiety of β-glucogallin extends into the “specificity” pocket and “anionic” pocket, potentially forming hydrogen bonds with Ser302 and obtaining favorable hydrophobic interactions with Leu300. These data suggest that β-glucogallin can bind to ALR2 as effectively

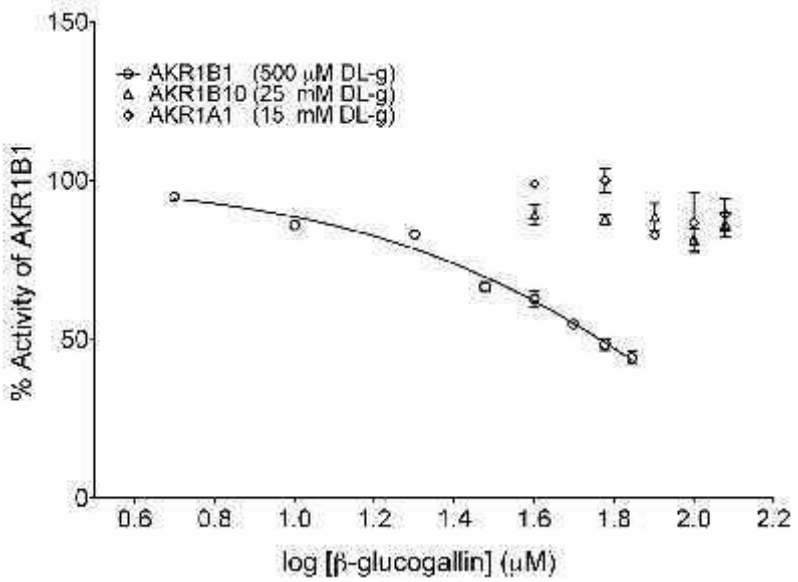
as sorbinil and potentially with greater affinity (Figure 4). β-glucogallin bound the active site of ALR2 obtaining a more favorable predicted binding energy than sorbinil (-44 kcal/mol and -32 kcal/mol, respectively).

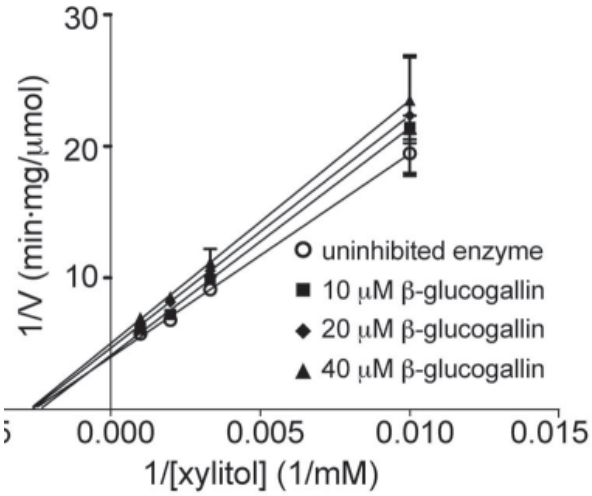
Figure 1. The elucidation of -glucogallin as an active component and inhibitor of ALR2 from *E. officinalis.*



(A) HPLC trace of pooled Sephadex® LH-20 fractions (B) The ^{1}H NMR and ^{13}C spectrum of the abundant active fraction from HPLC purification C) LC/MS/MS data indicate a peak at 687 *m/z* as a [2M aggregate + Na]

Figure 2. A: Enzyme inhibition studies with -glucogallin, ALR2 and other members of the AKR family. Enzyme activity was normalized to activity under the same conditions in the absence of β-glucogallin.





B: Lineweaver-Burk plot of the same data, depicting increasing slope and y-intercept characteristic of noncompetitive inhibition.

Figure 3. -glucogallin prevents sorbitol accumulation in transgenic (TG) human ALR2 expressing lenses *ex-vivo*. $P < 0.05$ (*).

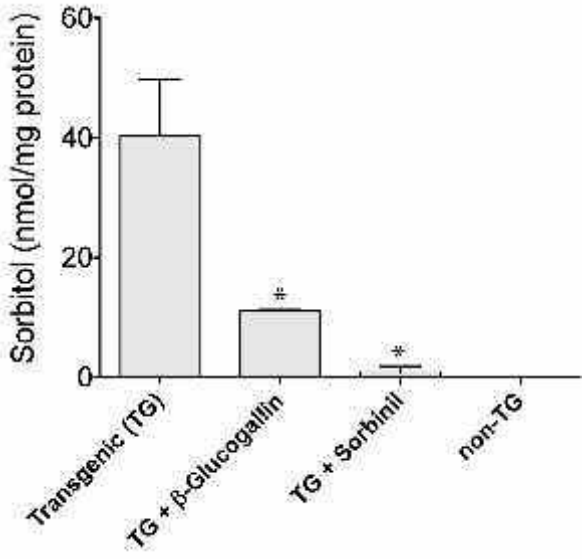
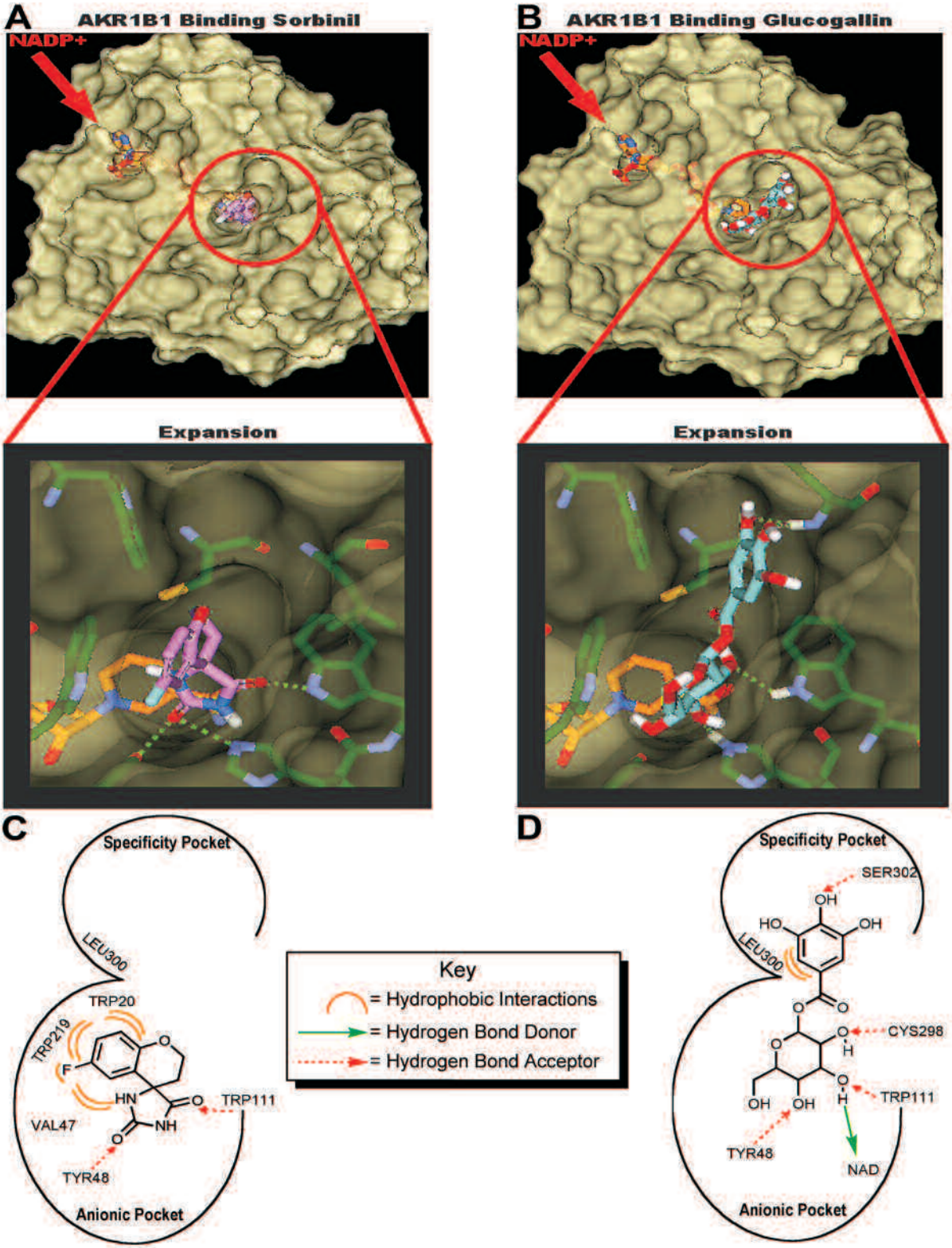


Figure 4 Computational modeling studies with -glucogallin and ALR2. Modeling comparison between sorbinil (A) and β-glucogallin (B) showing ligands bound to ALR2. The solvent accessible surface depicts the active site anionic and specificity binding pockets, with expansion images to emphasize the detail of each pocket. The active site interactions are summarized for sorbinil (C) and β-glucogallin (D).



Summary

Overall, these data confirm the initial studies, which showed the presence of an ALR2 inhibitor in the crude extracts from *E. officinalis*. In addition, the results indicated a high degree of selectivity of β-glucogallin for ALR2 over the most abundant other members of the human AKR family.

CONCLUSION

Natural products such as β-glucogallin are attractive as therapeutic leads in the treatment of diabetic complications. Since, their low toxicity allows them to be used as long-term prophylactics. In this context, The promise of dietary sources of aldose reductase inhibitors to prevent diabetic complications in animals had been investigated. Thus far, the most active ALR2 inhibitor from *E. officinalis* as β-glucogallin have successfully identified and purified and have demonstrated its inhibitory efficacy in vitro and in lens tissues in an ex-vivo model. Finally, this study supports the continued development of β-glucogallin as a therapeutic treatment against diabetic complications.

10 IMPACT OF AGENTS WITH POTENTIAL USE IN FUNCTIONAL FOODS ON BIOMARKERS FOR INDUCTION OF AGE RELATED DISEASES: INHIBITION OF ALDOSE REDUCTASE BY ELLAGIC ACID AND GENTIANA EXTRACTS

Cancer and cardiovascular diseases associated with atherosclerosis and diabetes constitute the major causes of age related diseases. During the last decade there has been a dramatic increase in the incidence of atherosclerotic diseases as well as of diabetes also in the Indian population, a development that has been linked to changes in food habits. However, in India insufficient means have been allocated to this newly emerging health problem. A number of epidemiological studies have consistently demonstrated the protective effects of fruits and vegetables with respect to age related diseases such as diabetes, atherosclerosis and several types of cancer. In Europe, this association seems especially obvious with respect to the protective action of the traditional Mediterranean-style diet, and where antioxidant constituents in red wine also appear to play a significant role. High consumption of fish rich in long chain omega-3 fatty acids has, likewise, been found to protect against cardiovascular disease, and not unexpectedly, life expectancy in Iceland is considerably higher than in most other countries.

The crucial issue that this project attempts to tackle is the following: While beneficial effects have been firmly established, it has not been possible to identify exactly which components in food are mainly responsible for the protective action with respect to age-related diseases, mainly because of the complex exposures involved, as well as the interaction with genetic and other confounding factors. The aim of this project is to investigate the protective action of agents with potential use as functional food constituents with respect to cancer, diabetes and cardiovascular disease. In collaboration between EU and Indian research centers, the proposal features a multipronged approach, where the protective action of various non-toxic agents are studied *in vitro* as well as in rodent models with respect to induction of DNA lesions, tumors and biomarkers for the development of diabetes, diabetic retinopathy and atherosclerosis. We at the National Institute of Nutrition will focus on the potential of functional foods against diabetic complications by assessing their ability to induce or modulate biomarkers of diabetes or diabetic complications using *in vitro, ex vivo* and animal models and human cohorts.

Prolonged exposure to chronic hyperglycemia in diabetes can lead to various complications affecting the cardiovascular, renal, neurological, and visual systems. Although mechanisms leading to diabetic complications are not completely understood, many biochemical pathways associated with hyperglycemia have been implicated. Among these, the polyol pathway has been extensively studied. Experimental animal models suggest that the inhibition of ALR2 could be effective in prevention of certain complications. A number of ALR2 inhibitors (ARI) have been developed for diabetic complications; however, none of them has achieved worldwide use because of limited efficacy or undesirable side effects. Thus, there is a need for developing and evaluating new ARI with efficacy, selectivity, and safety.

Ellagic acid (EA; 2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione) is found in numerous fruits and vegetables and other plant foods. Previously we have shown that EA inhibited or prevented AGE formation under *in vitro* conditions. *Gentiana lutea* is a plant belonging to the family *Gentianaceae*, which grows in the grassy alpine and subalpine pastures of central and southern Europe. The roots of *G. lutea* are commonly consumed as a beverage in some of the European countries and are also known to have medicinal properties due to the presence of bitter glycosides.

Therefore, in the present study we have assessed the ARI potential of EA and various extracts of *G. lutea*. We also investigated the effects of EA and Gentiana extracts on intracellular sorbitol accumulation in red blood cells (RBC) under *ex vivo* high glucose conditions which reinforce the ARI potential of *Gentiana*. Further analysis was done with ellagic acid in lens organ culture and retinal explant culture.

METHODOLOGY

Aldose reductase (ALR2) assay: The assay was carried out as described previously [4]. The reaction was initiated by the addition of NADPH at 37ºC in absence and presence of different inhibitors (Ellagic acid or *G.lutea extracts*). The percent of inhibition with test compounds was calculated considering the ALR2 activity in the absence of the inhibitor as 100%. The concentration of each test sample giving 50% inhibition (IC_{50}) was determined by non-linear regression analysis of log concentration of extract versus percentage inhibition.

Molecular docking studies: Molecular docking (Ellagic acid and *G.lutea* constituents) was done by discovery (discover 2.7) package on an O2 (R12000) workstation. All ligands were minimized and least energy conformations were taken for docking studies. Crystal structure of aldose reductase (human ALR2 or AKR1B1) was downloaded from Brookhaven data bank (PDB: 1PWM) and protein structure minimized by using charmM force field. All water molecules were removed. Docking was done by discovery ligandfit module in a protein created sphere of about 12 Å around the active site. After docking, poses were viewed by DS viewer and Biosolve IT Poseview.

Sorbitol accumulation in RBC: Five mL blood was collected from healthy male volunteers on overnight fasting in heparinized tubes. Red blood cells were separated by centrifugation and washed thrice with isotonic saline. Washed RBC were suspended in Kreb's-ringer bicarbonate buffer, pH 7.4 (pre-equilibrated with 5% CO_2) and incubated at 37°C in presence of 5% CO_2 for 3 h under normal (5.5 mM) and high glucose (55 mM) conditions [4]. The effect of compounds *(*Ellagic acid, *G.lutea extracts)* on sorbitol accumulation was evaluated by incubating RBC with different concentrations.

***Estimation of sorbitol in RBC*:** At the end of incubation period, RBC was homogenized in 9 volumes of 0.8 M perchloric acid. The homogenate was centrifuged at 5,000xg at 4°C for 10 min and the pH of the supernatant was adjusted to 3.5 with 0.5 M potassium carbonate. The sorbitol content of the supernatant was measured by fluorometric method as described previously using a spectrofluoro-meter.

Eye lens organ culture studies: Three-month-old male Wistar rats were sacrificed by cervical dislocation and eyes were enucleated immediately. Lenses were dissected by posterior approach and cultured in modified M-199 medium containing antibiotics and 30 mM glucose in the absence and presence of 100 and 200 μM ellagic acid for a period of 18 h (for sorbitol estimation) and 5 days (for lens morphology) using a method described previously. Retina from the above eye balls were also cultured under similar conditions for 18 h and terminated for sorbitol estimation. After incubation, lenses and retina were homogenized in 20 mM sodium phosphate buffer. The soluble fraction was separated from insoluble protein by centrifugation at 15000×g and the protein concentration was estimated. ALR2 activity was measured in total soluble fraction and estimation of sorbitol accumulation was done in total homogenate. Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the IAEC (Institutional Animal Ethical Committee).

RESULTS

a. Inhibition of aldose reductase by ellagic acid

- Ellagic acid (EA) inhibited rat and human ALR2 with IC_{50} values of 68.5 ± 3.3 and 46 ± 4 nM respectively (Figure 1).
- EA inhibited human recombinant ALR2 in a noncompetitive manner (Table 1), but was found to be a poor inhibitor of closely related members of the aldo-keto reductase (AKR) superfamily, particularly aldehyde reductase (ALR1).
- Molecular docking studies predict that EA forms a hydrogen bond with Ser-302 and participates in hydrophobic interactions with Trp-219. It appears that EA might bind to ALR2 in a closed type of conformation because it did not form any hydrogen bond with Leu-300 (Figure 2).
- Incubation of RBC with 55 mM glucose resulted in the accumulation of sorbitol about six-fold higher than the control, whereas presence of EA under high glucose conditions

Table 1. Kinetics of human recombinant AR in the absence and presence of ellagic acid

Ellagic acid (nM)	K_m (mM)	V_{max}
0	0.09±0.011	0.045±0.019
25	0.088±0.07	0.031±0.014
60	0.088±0.08	0.029±0.012
90	0.0874±0.05	0.023±0.01

Data are mean ±S.E. (n=3).
V_{max} is reported as µmoles NADPH oxidized/min/mg protein

Figure 1. Representative inhibition plots of ellagic acid against rat (*left panel*) and human ALR2 (*right panel*). Enzyme activity in the absence of ellagic acid was considered as 100%.

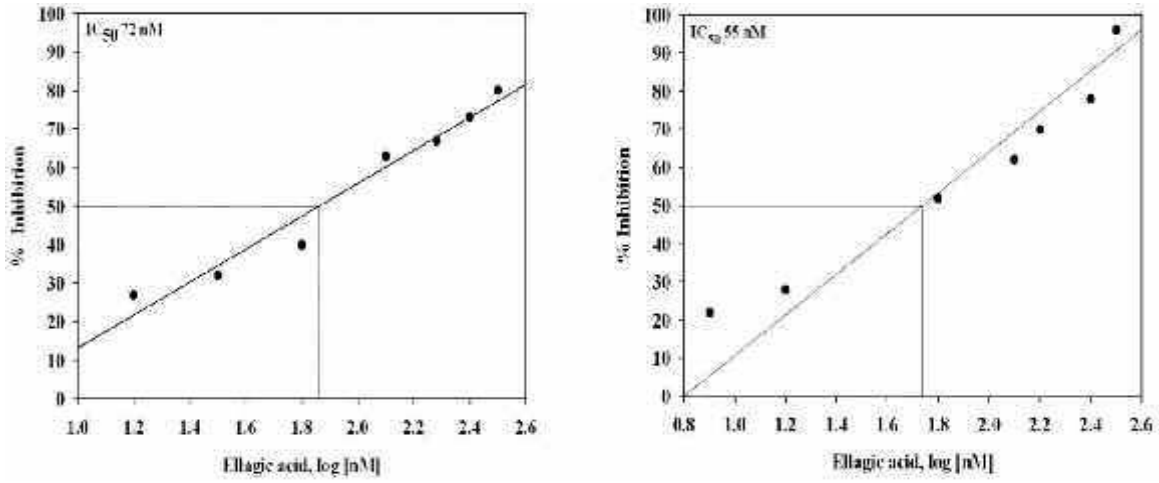
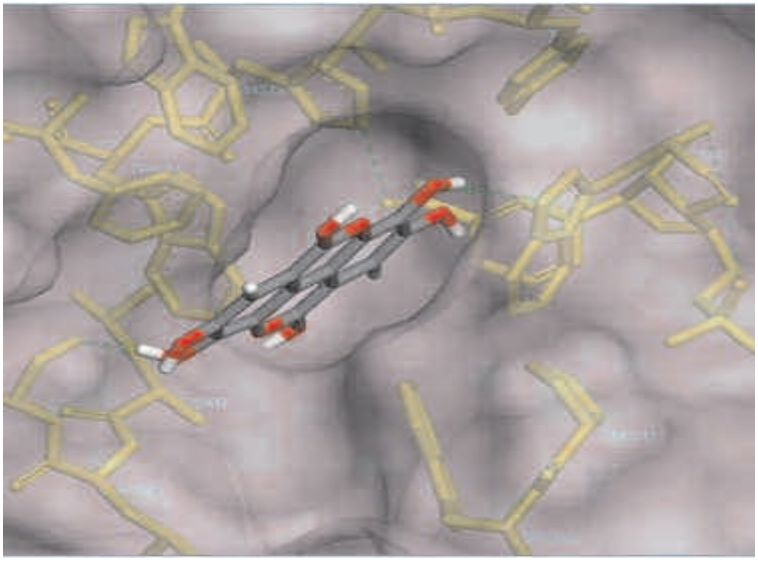
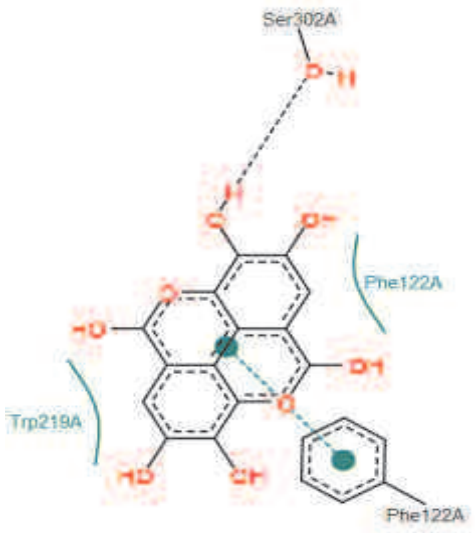


Figure 2. Stereoview of AKR1B1 docked with ellagic acid. (a) Ellagic acid docked into the active site and extended towards hydrophobic pocket.(b) Ellagic acid docked into active site depicts its hydrogen bond interaction with Ser-302 (dotted line) and hydrophobic interactions with Phe-122 (green dotted line)





lead to reduction in the accumulation of intracellular sorbitol in a dose dependent manner (Table 2).

- Incubation of rat lenses with 30 mM glucose resulted in the loss of transparency (opacification) in a time-dependent manner as assessed by the transmission of the light from the grid through the lens (Figure 3). It was observed EA prevented high glucose induced-lens opacity in a dose dependent manner until day 5. More importantly, activation of ALR2 and increased sorbitol levels due to hyperglycemic stress was prevented when lenses and retina were incubated with 30 mM glucose in the presence of EA (Figure 4).

Table 2. Effect of ellagic acid on intracellular red cell sorbitol level.

Group	Ellagic acid
Control	2.53±0.15
Glucose 55 mM	12.24*±0.75
Glucose 55 mM + 0.1 µM EA	10.16±0.51
Glucose 55 mM + 1 µM EA	7.16#±0.21
Glucose 55 mM + 10 µM EA	4.65#±0.23

Sorbitol levels are expressed as µg/ml RBC. * Indicates a statistically significant difference from the control group and # indicates a statistically significant difference from the glucose 55 mM group ($P<0.05$). Values are mean± SE of three independent experiments.

b. Inhibition of aldose reductase by *G.lutea* extracts

- The ether and methanol extracts of Gentiana showed greater inhibitory activities against both rat lens and human ALR2, the water and ethanol extracts showed moderate inhibitory activities (Table 3).
- Incubation of RBC with 55mM glucose resulted in the accumulation of sorbitol about six-fold higher than the control, whereas presence of all the extracts of *G. lutea* under high glucose conditions leads to reduction in the accumulation of intracellular sorbitol in a dose-dependent manner (Table 4).
- Molecular docking studies were carried out with commonly known constituents of *G. lutea*. For this, thirteen different compounds selected from various reported studies were used.

Table 3. IC_{50} values for ALR2 inhibition by *G. lutea* extracts

Values are mean ± standard deviation of three independent experiments

Name of the extract	Rat ALR2 (in µg)	Recombinant ALR2 (in µg)
Water extract	260 ± 20	70 ± 15
Ethanol extract	114 ± 12	82 ± 20
Methanol extract	112 ± 15	23 ± 5
Ether extract	79 ± 12	36 ± 5

Figure 3. Rat lenses were cultured in modified M-199 medium at different time points. **(A)** Lens in the medium alone, **(B)** in the presence of 30 mM glucose, **(C)**30 mM glucose with 100 μM EA, (**D**) 30 mM glucose with 200 μM EA

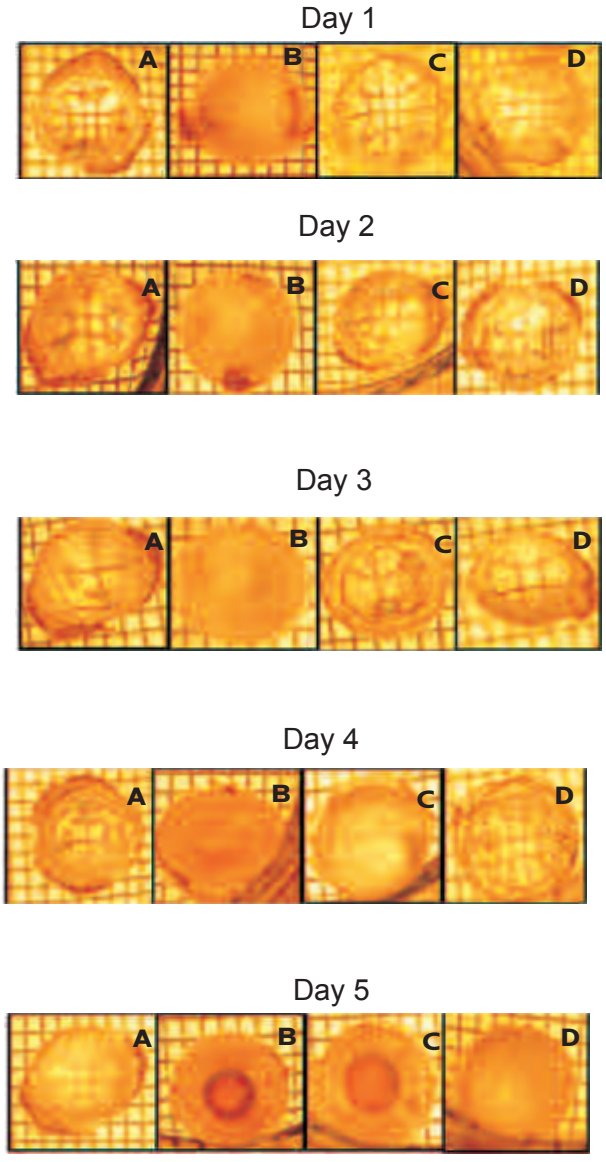


Figure 4. ALR2 activity (A) and sorbitol estimation in cultured rat lens (B) and retina (C). Culture conditions included (1) 5.5 mM glucose, (2) 30 mM glucose, (3) 30 mM glucose plus 100 μM EA, (**4**) 30mM glucose plus 200 μM EA

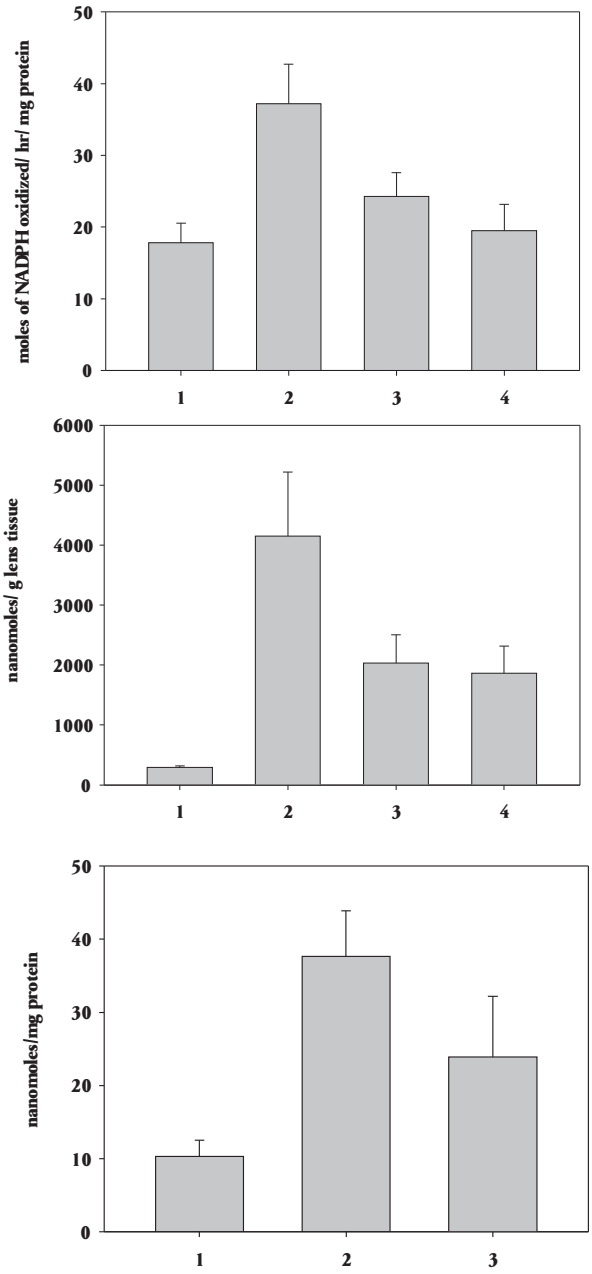


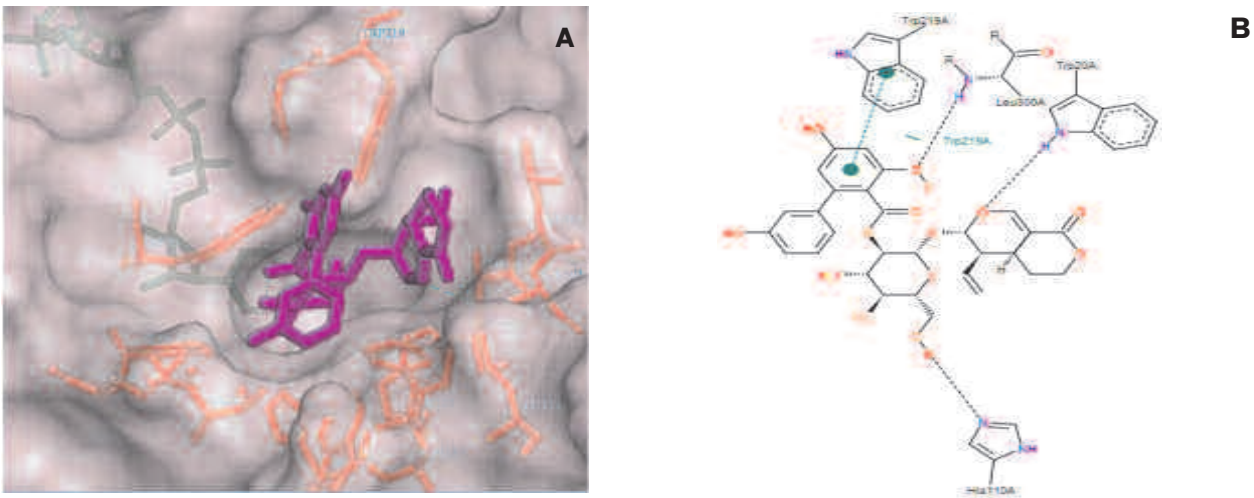
Table 4. Effect of *G. lutea* water, methanol and ether extracts on intracellular red cell sorbitol levels. Sorbitol levels are expressed as µg/ml RBC.

Group	Water extract	Methanol extract	Ether extract
Control	2.25±0.016	2.47±0.22	2.46- 0.25
Glucose 55 mM	12.77*±1.21	11.66*± 1.44	12.16*- 1.08
Glucose 55 mM + 10 µg	8.85#±0.25	8.52#± 1.04	7.25#- 0.65
Glucose 55 mM + 50 µg	---	7.43#±0.314	5.74#- 0.69
Glucose 55 mM + 100 µg	8.15#± 0.32	---	4.49#- 0.32

Sorbitol levels were measured in RBC incubated in the presence of normal (5.5 mM) and high (55 mM) glucose for 3h. * indicates a statistically significant difference from the control group and # indicates a statistically significant difference from the glucose 55 mM group (ANOVA, P<0.05). Values are mean± standard deviation of three independent experiments.

- Among the thirteen compounds, it was observed that amarogentin showed the highest dock score and possibly interacts with active site residues His-110, Trp-111, Leu-300, and Leu-301 (Figure 5). It appears that amarogentin might bind to ALR2 in an open type of conformation because of formation of hydrogen bond with Leu-300 (Figure 5).

Figure 5. *Panel A* Amarogentin docked into the active site of ALR2 and extended towards hydrophobic pocket. *Panel B* Amarogentin docked into active site of ALR2 and depicts its hydrogen bond interaction with residues Trp-20, His-110, Leu-300 (dotted line) and hydrophobic interactions with Trp-219 (green dotted line).



11 POTENTIAL ROLE OF DIETARY NUTRIENTS VITAMIN A AND POLYUNSATURATED FATTY ACIDS (PUFA) ON REGULATION OF DEVELOPMENT AND/ OR CONTROL OF OBESITY USING A GENETIC OBESE MUTANT RAT MODEL (WNIN/ Gr- Ob)–*NUTRIENT - GENE INTERACTIONS*

Obesity, the multi-factorial metabolic disease is spreading across the globe including developing countries like India. This condition can be defined as "accumulation of excess fat due to imbalanced energy metabolism/homeostasis". From the recent past, among the dietary factors, the role of RA or vitamin A on energy homeostasis has become the focus of intense research. Liver is the major organ involved in the storage and homeostasis of retinoids. However, adipose tissues contain substantial amounts of retinol and retinyl esters. Adipose tissue, the fat storing organ plays a major role in energy homeostasis by being the site for various enzymes involved in lipogenesis, carbohydrate metabolism and various cytokine and hormone synthesis. The present study was planned to address the impact of vitamin A supplementation on obesity and its associated disorders such as dyslipidemia and impaired glucose tolerance in WNIN/GR-Ob-obese rats.

AIM

The primary aim of this project was to understand the possible role of vitamin A (at the dose of 129mg/kg diet) in controlling body weight gain/obesity and its associated disorders using obese mutant rats with impaired-glucose tolerance trait; namely WNIN/GR-Ob strain.

OBJECTIVES

- To study the effect of chronic dietary high vitamin A supplementation on obesity/adiposity.
- To understand the role of vitamin A on hepatic and adipose tissue lipid metabolism.
- To study the regulation of various genes of lipid metabolism by vitamin A in both liver and adipose tissue.

Work done

Experimental design

Based on phenotypes, 180days old male rats of WNIN/GR-Ob strain were broadly divided into two groups (A & B)) consisting of 12 lean and 12 obese respectively. Then each group was further divided into two subgroups (I & II) consisting of 6rats from each phenotype. Subgroups A I and B I received stock diet containing 2.6mg vitamin A/kg diet, while A II and B II received vitamin A-enriched diet (129mg/kg diet) (Table 1). The stock diet and vitamin A-enriched diets are identical with regard to the nature and concentration of all ingredients except the quantity of vitamin A. The study design was approved by the Institutional Animal Ethical Committee. The animals were maintained on their respective diets for a period of 90days. At the end, experimental animals were killed after overnight fast, blood and various tissues were collected for further analyses.

Table 1. Experimental design

Experimental Groups			
Lean		Obese	
A-I	A-II	B-I	B-II
Stock diet (2.6mg vitamin A/ kg diet)	Vitamin A-enriched diet (129mg/kg diet)	Stock diet (2.6mg vitamin A/ kg diet)	Vitamin A-enriched diet (129mg/kg diet)

METHODOLOGY

1. Gastrocnemus muscle (skeletal muscle) total lipid was extracted, analyzed for triglyceride (TG) and fatty acid composition.
2. Skeletal muscle glycogen content and protein tyrosine phosphatase 1B (PTP-1B) activity were determined by enzymatic method.
3. Skeletal muscle gene expression studies were performed using quantitative real time PCR (qRT-PCR) as per standard procedures and protocols.
4. Values are expressed as mean±SEM (4rats of each group). Stock diet Vs Vitamin A-enriched diet groups were compared and P<0.05 level was considered significant.

RESULTS

1. Impact of vitamin A on muscle TG and glycogen levels and PTP-1B activity

Skeletal Muscle, triglyceride content was significantly increased in obese rats fed on vitamin A-enriched diet as compared to that of obese rats receiving stock diet containing normal vitamin A level i.e. 2.6mg/kg diet. However, lean rats did not show such response to high vitamin A-diet feeding (Table 1).

Paradoxical effect of vitamin A on glycogen levels was observed in lean and obese rats. In the former, glycogen levels were significantly decreased, while in the latter, significantly increased as compared with their respective controls fed on stock diet. Further, muscle PTP-1B activity remained unaltered by vitamin A-enriched diet (Table 1).

Table 1. Impact of vitamin A on muscle TG and glycogen levels and PTP-1B activity

Muscle parameters	LEAN		OBESE	
	A-I	A-II	B-I	B-II
Triglycerides (mg/g)	3.3±0.51	4.0±1.27	4.7±0.65	11.0±0.93*
Glycogen (ug/g)	154±23.5	30±2.4*	155±29.1	276±51.9*
PTP-1B (%)	100.0±2.96	103.0±6.56	115.0±6.36	141.0±7.15

Values are expressed as mean ± SEM of 4rats of each group. Stock diet Vs Vitamin A enriched diet groups were compared and P<0.05 level was considered significant (Oneway ANOVA with LSD)

2. Impact of vitamin A on muscle fatty acid composition

In general, vitamin A supplementation did not alter the fatty acid composition in lean rats, while in obese rats it significantly increased the monounsaturated fatty acid; oleic acid (C18:1) and decreased the long chain polyunsaturated fatty acids, namely arachidonic acid (C20:4), docosapentaenoic acid (22:5) and docosahexaenoic acid (22:6) levels, as against obese rats fed on stock diet (Table 2).

3. Impact of vitamin A on muscle gene expressions by Quantitative Real Time PCR (qRT-PCR)

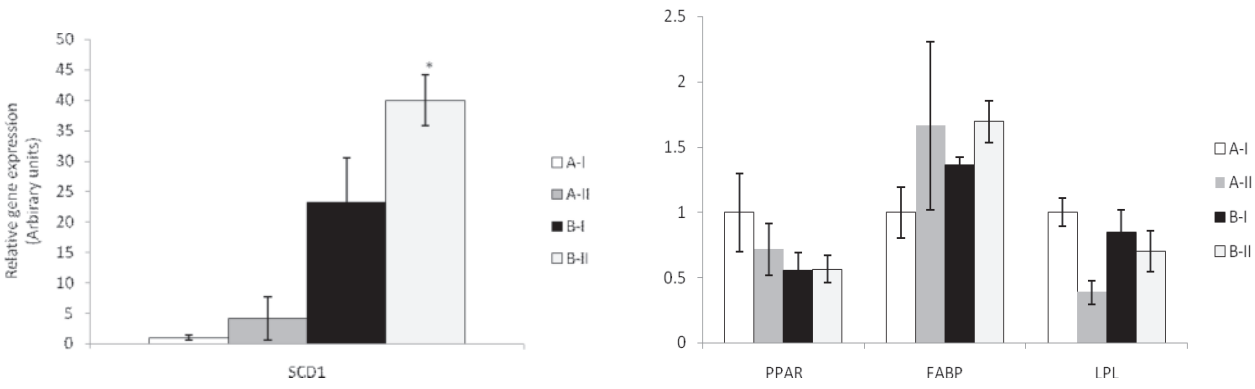
Compared to stock-diet fed obese rats, altered expression of various important lipogenic/fatty acid oxidation pathway genes were observed in vitamin A challenged lean and obese rats, Stearoyl CoA desaturase1 (SCD1) level significantly increased in vitamin A-enriched diet fed obese rats, while fatty acid binding protein (FABP), lipoprotein lipase (LPL) levels and peroxisome proliferator activated receptor α (PPAR α) expressions remained unaltered. On the contrary, LPL mRNA levels significantly decreased in lean rats receiving vitamin A-enriched diet as compared to those of stock diet-fed lean rats (Figure 1a).

Table 2. Impact of vitamin A on muscle fatty acid composition

Fatty acid (%)	LEAN		OBESE	
	A-I	A-II	B-I	B-II
C 14:0	1.5 ± 0.21	1.2 ± 0.18	1.5 ± 0.01	2.3 ± 0.47
C 14:1	ND	ND	ND	1.7 ± 0.51
C 16:0	22.8± 0.96	21.5 ± 1.40	22.3 ± 0.67	19.9 ± 0.89
C 16:1	2.07 ± 0.37	3.40 ± 1.16	7.2 ± 1.28	10.5 ± 1.15
C 18:0	12.7 ± 0.92	10.8 ± 1.56	8.0 ± 1.59	4.1 ± 0.93
C 18:1	19.7 ± 1.95	25.3 ± 3.80	23.6 ± 3.47	35.2 ± 1.85*
C 18:2	23.8 ± 1.41	25.1 ± 3.05	22.6 ± 1.24	24.9 ± 0.84
C 20:0	0.9 ± 0.3	ND	ND	0.7 ± 0.5
C 18:3n3	ND	1.1 ± 0.03	1.2 ± 0.6	0.4 ± 0.29
C 20:1	ND	0.4 ± 0.02	0.9 ± 0.44	ND
C 20:3	0.9 ± 0.09	0.9 ± 0.18	1.0 ± 0.11	0.7 ± 0.06
C 22:0	0.2 ± 0.03	0.2 ± 0.03	0.4 ± 0.11	0.3 ± 0.14
C 20:4 n6	9.8 ± 0.61	7.3 ± 1.03	5.1 ± 0.94	1.7 ± 0.25*
C 20:5 n3	0.7 ± 0.10	0.6 ± 0.13	ND	ND
C 24:0	0.2 ± 0.018	0.2 ± 0.03	0.3 ± 0.12	0. 2 ± 0.09
C 22:5 n3	1.1 ± 0.23	0.9 ± 0.16	0.9 ± 0.20	0.4 ± 0.09*
C 22:6 n3	2.8 ± 0.19	1.9 ± 0.28 [a]	1.3 ± 0.28	0.4 ± 0.08*
?SFA	37.8 ± 2.04	33.9 ± 3.14	31.8 ± 2.24	27.5 ± 2.03
?Mufa	22.2 ± 2.24	28.9 ± 4.54	36.6 ± 4.47	44.5 ± 1.98
?LCn 6	10.7 ± 0.67	8.2 ± 1.19	6.17 ± 0.93	2.43 ± 1.58
?LCn 3	4.6 ± 0.35	3.2 ± 0.45	2.4 ± 0.53	0.8 ± 0.13

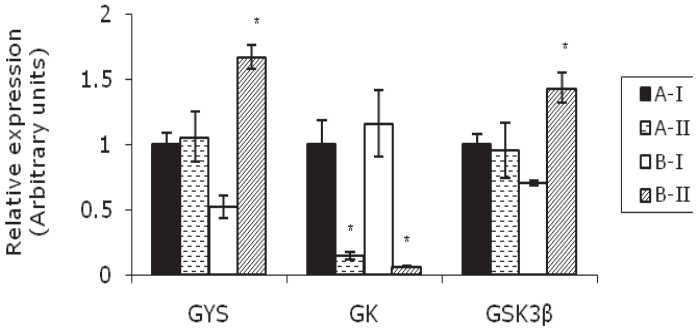
Values are expressed as mean ± SEM of 4rats of each group. Stock diet Vs Vitamin A enriched diet groups were compared and P<0.05 level was considered significant (Oneway ANOVA with LSD)

Figure 1a. Impact of vitamin A on gastrocnemus muscle gene expressions



Compared to lean rats, basal expression levels of glycogen synthase and glycogen synthase kinase 3β genes were significantly lower in obese rats. However, by feeding vitamin A-enriched diet, the levels were elevated significantly as compared to their stock diet-fed obese rats. On the contrary, glucose oxidative pathway gene glucokinase (GK) mRNA levels were reduced significantly by vitamin A-enriched diet fed lean and obese rats as compared to their respective stock diet-fed counterparts (Figure 1b).

Figure 1b. Impact of vitamin A on gastrocnemus muscle gene expressions



Values are expressed as mean ± SEM of 4rats of each group. Stock diet Vs Vitamin A enriched diet groups were compared and P<0.05 level was considered significant (Oneway ANOVA with LSD).

CONCLUSION

Over all, long-term consumption of vitamin A-enriched diet improves muscle insulin sensitivity in obese rats as indicated by increased glycogen content possibly through glycogen synthase transcriptional regulation.

12 ABDOMINAL OBESITY AND ITS RELATION TO PLASMA HOMOCYSTEINE AND OTHER CHD RISK FACTORS IN MIDDLE AGED MEN

High incidence of CHD and increased prevalence of coronary heart disease risk factors have been reported from several urban areas of India. The higher risk of CHD in migrated Indians as well as in native Indians was also not fully explained by the conventional risk factors alone. Moderately elevated plasma homocysteine concentrations have been considered as an independent risk factor for CHD, and the values are also reported to be higher in Indians. Recently, it has been reported that high plasma cysteine concentrations are more associated with several metabolic disorders. In addition to the known biochemical CHD risk factors, Indians had a phenotypic paradox i.e. at lower BMI levels Indians had high % of body fat and high waist circumference (WC). However, WC has been considered as a good measure to define abdominal obesity, and the values are not uniformly accepted to all populations. Among all the urban cities, Hyderabad is one of the rapidly growing cities in south India. Urbanization results in influence on both dietary and lifestyle factors leads to, high prevalence of diabetes and its associated CHD risk in this population. Even though the prevalence of CHD and its risk factors distribution is well reported from northern and western cities, it was poorly addressed among those residing in Hyderabad.

HYPOTHESIS

Hyperhomocysteinemia is one of the risk factors in abdominal obesity in addition to other CHD risk factors in middle aged men.

OBJECTIVES

- To study the association of plasma homocysteine with anthropometric (BMI, WC) and body composition measures in urban (Hyderabad) middle aged men.

- To examine the distribution of other CHD risk factors, among different BMI, WC and body composition in relation to plasma homocysteine.
- To study the distribution of plasma aminothiols such as cysteine, cysteinyl glycine, homocysteine and glutathione levels in urban middle aged men.

METHODOLOGY

Study design

As per the sample size requirement, 300 apparently healthy middle aged (35-55 years) men were recruited in different BMI and WC at NIN (National Institute of Nutrition), Hyderabad. Subjects were from in and around areas, excluding the subjects, who were diagnosed with diabetes.

Anthropometric and body composition measurements

Standing height was measured to the nearest 0.5cm using a wall-mounted stadiometer and body weight was measured to the nearest 0.1kg in an automated weighing machine. WC was measured at the minimum circumference between iliac crest and the rib cage in standing position, hip circumference was measured in minimal clothing using tape. BMI was determined as Quetelet's index (kg/m^2). Modified Asian criteria was used to define obesity ($\geq$25 BMI), and abdominal obesity (WC$\geq$90 cm). Percent of body was measured using skin fold thickness measurement method. Skin fold thickness at 4 sites such as triceps, biceps, sub scapular and suprailiac was measured using Harpenden calipers. Percent of body fat was calculated using Siris equation. % of Body fat = (4.95/ density - 4.5) ×100. Blood pressure was measured on the left arm with the use of BP apparatus spignomanometer twice after five minutes of rest in a quiet room. Modified Asian criteria were used to define obesity ($\geq$25BMI) and abdominal obesity (>90 cm WC).

Diet and lifestyle factors

Information was collected from the participants about their frequency of consumption of food groups over the previous year by choosing from responses coded 1-5. Intake of mutton, fish, green leafy vegetables and whole grains are chosen for inclusion as rich sources of B vitamins. Exercise, a range of activities including leisure time activities and nature of work were further categorized according to time spent and intensity. Diet history (vegetarian vs. omnivores) and lifestyle factors such as smoking, alcohol consumption were also recorded.

Biochemical measurements

About 5ml of overnight fasting blood was collected into EDTA tubes and plasma was separated and stored at –70°C for the measurement of the following biochemical parameters.

1. *Plasma Lipids:* Total cholesterol, triglycerides and HDL-cholesterol were measured by enzymatic kit method (Biosystems). LDL- cholesterol was calculated using FreidWalds-formula).
2. Plasma glucose was measured using enzymatic kit (Biosystems) method.
3. Plasma hsC - reactive proteinwere measured by ELISA kit method.
4. Plasma insulin levels were measured by Radio immunoassay (RIA) supplied by BRIT.
5. Plasma amino thiols were reduced with tris-(2-carboxyethyl) phosphine hydrochloride, followed by protein precipitation with trichloroaceticacid, free thiols were derivatized with SBD-F. Plasma thiols were separated and measured by HPLC with fluorescence detection. Samples were spiked with standards and recoveries were 97%.
6. HOMA-IR was calculated using plasma glucose and insulin values using the formula Plasma glucose in mmoles/l×insulin(µU/ml)/22.5.
7. Hyperhomocysteinemia was defined as >15µmoles/L.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS version 14.0 for WINDOWS (SPSS Inc, Chicago, IL). Results were expressed as mean±S.D. One-way ANOVA was utilized for comparison of different biochemical CHD risk factors between different obesity measures. One-way ANOVA/'t' test were utilized for comparing the biochemical CHD risk factors between different quartiles of BMI and also abdominal obesity. Associations of biochemical CHD risk factors with dietary variables were tested by Chi-square. Pearson's and Spearman's correlation coefficients were calculated between anthropometric (BMI, WC & WHR), body composition (% of body fat) indices, plasma tHcy and other biochemical CHD risk factors. These correlations were examined with 't' test for the significance of the relationship. Statistical significance was set at a p value<0.05.

RESULTS

Mean age of the present study group men was 45.06±6.56 years. Prevalence of some of the CHD risk factors are given in table 1. As per the modified Asian criteria, 50% of the present study group men were obese (≥25 BMI) and 45% were abdominally obese (≥90cm Waist circumference). Other obesity measures such as waist to hip ratio (≥0.9) and % of body fat (>25%) have shown higher prevalence (70% and 66% respectively) compared to obesity and abdominal obesity. Of the conventional CHD risk factors, prevalence of hypertension was 40% in the present study group men. Prevalence of hypertension was significantly higher in all the four obesity measures BMI≥25, WC≥90cm, WHR≥0.9, &% of body fat≥25 (53%, 58%, 47% & 53% respectively).

With respect to plasma lipid concentrations, lower HDL- cholesterol (<35mg/dl) was shown to be highest (50%) followed by total cholesterol (24%), LDL- cholesterol (32%) and triglycerides (16%). Among the lipid concentrations, mean total cholesterol concentrations were significantly ($p<0.05$) higher (181.6mg/dl) in high (≥25) BMI compared to normal (<25) BMI (172.0 mg/dl) and were also significantly ($p<0.01$) higher 181.8 mg/dl in high % of body fat (≥25) compared to normal body fat (<25) 168.6mg/dl. Mean plasma triglyceride (TG) concentrations were significantly ($p<0.05$) higher 117.4 mg/dl in obese (≥25BMI) group compared to normal (<25) BMI group 103.7mg/dl, and TG concentrations were also significantly ($p<0.05$) higher 118.6mg/dl in high WHR (≥0.9) compared to normal WHR (<0.9) 102.9 mg/dl. Mean HDL- Cholesterol concentrations did not show any significant difference between any of the obesity measures. However, mean LDL- cholesterol concentrations were found to be significantly ($p<0.01$) higher 122.3mg/dl in high % of body fat (≥25) compared to normal body fat (<25)112.6 mg/dl. Mean plasma glucose concentrations were shown to be were significantly ($p<0.01$) higher 104.4mg/dl only in high WHR compared to normal WHR 88.4mg/dl. Prevalence of hyperhomocysteinemia (≥15µmoles/ liter) was 28% in the present study group men.

Of the 20 % prevalence of vegetarians in the preset study group, 42% men had hyperhomocysteinemia. Vegetarian men had significantly ($p<0.001$) higher (19.02± 21.04µmoles/L) homocysteine concentrations compared to non-vegetarian men (11.36 ± 8.96 µmoles/L).

Table 1. Prevalence (%) of some of the CHD risk factors in middle aged men (n=300)

BMI = 25 (kg/m2)	50
WC = 90 (cm)	45
WHR = 0.9	70
% of body fat = 25	66
Hypertension	40
Total Cholesterol (= 200mg/dL)	24
HDL-Cholesterol (< 35mg/dL)	50
TG (= 150 mg/dL)	16
LDL-Cholesterol (= 130mg/dL)	32
Insulin (=30µU/mL)	20
HOMA-IR (= 4.0)	25
hs-CRP (= 3mg/L)	23
Hyperhomocysteinemia (= 15µmoles/L)	28

Plasma homocysteine concentrations were not significantly different between any of the independent obesity measures such as ≥25 BMI, high WC (≥90 cm), high WHR (≥0.9) and high percent of body fat (≥25). As mentioned in the study design, at a normal range of BMI (≥23-25) men with high WC (≥90 cm) had higher (17.47±15.25μmoles/l) plasma homocysteine concentration than men with normal WC (12.92±7.96μmoles/l). In relation to other biochemical CHD risk factors, mean plasma homocysteine concentrations were significantly ($p<0.05$) higher (17.17±16.6μmoles/L) in low (<35mg/dl) HDL-Cholesterol group compared to high (>35mg/dl) HDL-Cholesterol group (13.47±13.05) μmoles/L.

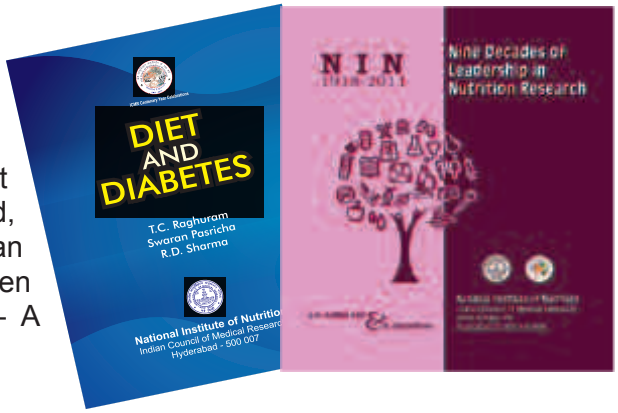
CONCLUSIONS

BMI and WC independently has not shown any significant difference in plasma homocysteine concentrations in middle aged men, however, with a significant difference in plasma homocysteine between normal and low HDL-cholesterol, a negative correlation ($r=0.247$; $p<0.05$) was found between plasma homocysteine and HDL- Cholesterol needs to be further confirmed in populations at risk for hyperhomocysteinemia.

VI EXTENSION AND TRAINING

A. SERVICE ACTIVITIES

1. PUBLICATIONS



The popular publication "Diet and Diabetes" was revised and reprinted. A new publication "NIN 1918-2011: Nine decades of leadership in Nutrition Research" was brought out during the last year. The other publications which were reprinted, on popular demand include Nutritive Value of Indian Foods, Dietary Guidelines for Women and Children (English) and Dietary Guidelines for Indians – A Manual.

2. TRAINING PROGRAMMES

Regular Training Programmes:

This year a total of thirty six candidates have attended the regular training programmes of the Institute viz. (i) MSc (Applied Nutrition) III Batch 2012-13: 17 participants (ii) Post-Graduate Certificate Course in Nutrition: 9 participants (iii) Annual Training Course on Endocrinological Techniques and their Applications : 10 participants.

The Mini-Convocation for the 2nd Batch of MSc (AN) was held on Dec. 5, 2012 certificates were awarded to the successful candidates and also Dr.B.K.Nandi Fellowships and Prize was given to the meritorious students.

3. EXTENSION ACTIVITIES

3.1 Exhibitions

- Arranged Institute's stall at ELDERS' MELA organized by Help Age India and Red Cross at Zoroastrian Club, Secunderabad. His Excellency Shri ESL Narasimhan, the Governor of Andhra Pradesh visited the NIN stall during the Exhibition (Oct. 2-4).
- Organised Nutrition Awareness stall at '*Bharat Nirman*', Public Information Campaign, organized by Press Information Bureau, Ministry of Information and Broadcasting, Govt of India, at Mancherial, Adilabad District (Dec. 15-17).

- Represented NIN in the ICMR's poster gallery at the 100th Indian Science Congress, Bhuvaneswar. Delivered a lecture on "Importance of educational outreach activities on nutrition and health" in the Workshop organized on 'Outreach Activities' by Indian Science Congress (Jan. 3-7).

3.2 Popular Lectures/Awareness Camps

- Organized Nutrition Education Sessions for bus drivers during a special program, organized by APSRTC, at various APSRTC bus depots in Hyderabad (July 23-29).
- A talk on the theme with special emphasis on Nutrition in Adolescent and Women's Health at St. Francis College for Women, Begumpet, Hyderabad. Over 100 undergraduate students and their faculty from Life Sciences Department participated (Sept. 3).
- Nutrition orientation programme at NIN for NGOs working at the community level. Themes concerning Nutritional Status of Indian Population, Anaemia Prevalence and its Health Impact, Recommended Dietary Allowances for Adolescent age were discussed. Prominent NGOs World Vision, M.V.Foundation and Andhra Samithi participated in the programme (Sept. 4).
- A Nutrition Awareness Programme in collaboration with Andhra University at Waltair, Visakhapatnam.

3.3 Radio Talks and TV Programmes

- Delivered Radio Talks on the topics "Low cost nutritious foods", "Importance of nutrition in a life style approach" during National Nutrition Week celebrations (Sept. 4, 7).
- Participated in the discussion on diabetes as part of the World Diabetic Day Celebrations in the Prime Time slot organized by Sakhsi TV. Discussed about the extension and education activities of NIN during the programme.

4. SPECIAL EVENTS

4.1 National Nutrition Week Celebrations (Sept 1-7, 2012)

In connection with the National Nutrition Week celebrations, the following programmes were organized:

- A talk on the theme with special emphasis on Nutrition in Adolescent & Women's Health at St. Francis College for Women, Begumpet, Hyderabad. Over 100 undergraduate students and their faculty from Life Sciences Department participated (Sept. 3).
- Nutrition orientation programme at NIN for NGOs working at the community level. Themes concerning Nutritional Status of Indian Population, Anaemia Prevalence and its Health Impact, Recommended Dietary Allowances for Adolescent age were discussed. Prominent NGOs World Vision, M.V.Foundation and Andhra Samithi participated in the programme (Sept. 4).
- A Nutrition Awareness Programme in collaboration with Andhra University at Waltair, Visakhapatnam (Sept. 4).
- Radio Talks on the topics "Low cost nutritious foods", "Importance of nutrition in a life style approach" (Sept. 4, 7).

- A one-day Workshop in association with Food and Nutrition Board on the theme of National Nutrition Week.

B. RESEARCH ACTIVITIES

1. Evaluation of nutrition reports based on research studies in leading Indian newspapers

The media is consistently ranked by the public as their primary source of nutrition and food information. Newspapers are an important source of health and nutrition information for many consumers. A large percentage of health reporting today deals with topics related to diet and fitness. Reporting on evolving diet and related health science issues presents a particular challenge for journalists as the public's thirst for this information grows and news reports influence daily food and life-style choices.

According to the International Food Information Council (IFIC), surveys show consumers rely more on the news media than on doctors and dieticians for food and nutrition information. Many newspapers report the findings of research studies published in peer-reviewed medical/ nutrition journals or reports released or papers presented in conferences. These reports act as bridge between scientific research and communications with the public. In a recent study conducted in India on coverage of nutrition-related topics by print media, it was observed at least 25% of news articles in English and vernacular dailies on nutrition-related issues were referring to findings of research papers published in peer reviewed journals.

It was also observed that, few of the news reports lacked consistency in presenting the research results to the readers. This could be attributed to variation in quality of reporting, which is determined by how accurately the scientific information is translated. The quality of reporting deals with factors such as accuracy, breadth and depth of reporting. Different studies have developed scores to assess the quality of such reports based on factors such as background information, description of study population, results, citation of original article, author details etc.

The current study was conducted to evaluate nutrition-related articles based on research reports in popular Indian newspapers. The objective of the study were to assess the quality of newspaper articles about nutrition research based on comparison with the original journal articles and also to compare the extent of accurate reporting of research among various dailies.

MATERIALS AND METHODS

Study Design

Prospective study for a period of six months from 1st September 2010 to 28th February 2011.

Sample

Selective sampling based on the daily circulation figures provided by the Audit Bureau of Circulation (ABC), India. Accordingly, the top three popular newspapers in English and Telugu – Times of India, The Hindu, Deccan Chronicle (English); Eenadu, Saakshi and Andhra Jyothi (Telugu) were selected for the study.

Sample Size

About 179 issues of six newspapers totaling 1074 issues during the study period of six months were considered for the study. Only those articles/ reports related to nutrition, food, dietetics that was based on findings of scientific research studies was considered. The inclusion and exclusion criteria for the samples are as follows:

Inclusion: Articles/ reports/ news items appearing in the newspapers quoting findings from research studies on nutrition, food and dietetics.

Exclusion: Advertisements, nutritional advice, recipes, editorials and other news items which may have description about nutrition and related issues, but do not contain any reference about the findings of any nutrition research.

Analysis

For this study, we looked at 10 variables regarding the reporting of nutrition-related research in six newspapers. We first examined whether the reader could locate the original study from information reported in the newspaper. For those articles for which the published study could be found, we also determined whether parts of the research were reported inaccurately or omitted and we compared reporting among newspapers. All the news reports were identified with manual search by the principal author. Newspaper articles were then matched with the appropriate journal report and rated on 10 items (Table 1). The following variables address the research methods and findings:

1. Sample size reported
2. Study population described
3. Description of variables measured in the study
4. study design and analysis described
5. Limitations of the study reported
6. Prime source of the report mentioned
7. Only secondary source of the report mentioned
8. News report based on a peer-reviewed study
9. Headline do not reflects the original study accurately
10. Appeared on Page-One

Each news report was scored as 0 for “no” or 1 for “yes” for each of the 10 variables. The maximum score was 10 points.

Statistical analysis

Descriptive statistics were calculated for all the ten variables of the study. Mean values of all these variables across the six newspapers were compared using *ANOVA*-F test with post Hoc tests of LSD (Least Significant Different) method. Level of significance was considered as 0.05.

RESULTS AND DISCUSSION

A total of 214 news reports on nutrition research were identified in all the six newspapers *viz.,* Deccan Chronicle, The Hindu, Times of India, Eenadu, Saakshi and Andhra Jyothi during the study period. Overall, vernacular (Telugu) newspapers published more number of reports (125) on

nutrition research compared to English dailies (89). Andhra Jyothi, stood top in publishing highest number (71) of nutrition research reports (table-1). Among the English dailies, Deccan Chronicle published 41 nutrition research reports followed by The Hindu (24) and Times of India (24) with equal number of reports. Saakshi daily published only 12 nutrition reports in the entire six months of the study period.

Table 1. Percentage of news articles containing each of 10 variables

S. No.	Variables in newspaper report	Deccan Chronicle n=41	The Hindu n=24	Times of India n=24	Sub total-1 n=89	Eenadu n=42	Saakshi n=12	Andhra Jyothi n=71	Sub total-2 n=125	Overall (Subtotal 1+2) N=214
1	Sample size reported	43.9	20.8	70.8	44.9	33.3	41.6	26.7	30.4	36.4
2	Study population described	46.3	25.0	70.8	47.1	30.9	41.6	29.5	31.2	37.8
3	Description of variables measured in the study	56.1	37.5	62.5	52.8	40.4	41.6	38.0	39.2	44.8
4	Study design and analysis described	65.8	33.3	66.6	57.3	40.4	50.0	53.5	48.8	52.3
5	Limitations of the study reported	21.9	4.1	16.6	15.7	0	0	0	0	6.5
6	Prime source of the report mentioned	48.7	29.1	70.8	49.4	14.2	33.3	12.6	15.2	29.4
7	Only secondary source of the report mentioned	51.2	70.8	29.1	50.5	85.7	66.6	87.3	84.8	70.5
8	News Report based on a peer-reviewed study	34.1	25.0	45.8	34.8	9.5	33.3	12.6	13.6	22.5
9	Headline do not reflects the original study accurately	2.4	0	0	1.1	0	8.3	5.6	4.0	2.8
10	Appeared on Page one	2.4	0	0	1.1	0	8.3	0	0.8	0.9

In all the six daily newspapers combined, more than 70% of the nutrition research reports revealed only secondary source of the information and less than 30% were based on the primary source. Reports based on secondary source were mostly quoted only the names of the country or University where the research was carried out or Representatives of food or pharmaceutical industries or foreign news papers like The Daily Mail, New York Times, and Washington Post as their source of information.

This indicates that, the nutrition research reports appeared in the studied news papers were mostly not extracted from the original (primary) source. It was observed that, all the reports based on primary source of information are not peer-reviewed studies. Only 22.5% of reports on nutrition research appeared in all the six dailies were based on peer-reviewed Journals. Of which English newspapers share two-third and Telugu dailies share only one-third reports. Few reports in the study were based on the claims made by even public relations professionals of food and pharmaceutical industries. A significant variation appeared between English and Telugu dailies pertaining to the source of information is that, of 89 nutrition research reports appeared in all the three English newspapers, 44 (49.4%) were based on primary source and 45 (50.6%) reports were quoted only secondary source of the information. Whereas, in all the three Telugu dailies only 29.4% reports mentioned primary source and the rest 70.6% were disclosed only secondary source of information.

Basic characteristic of any scientific research is its limitations. Each and every research or study usually has its own limitations. This vital aspect is missing in the newspapers reports on

Table 2. Percentage of news reports containing variables (from 1 to 8)

S. No.	News Paper	7 variables reported	6 variables reported	5 Variables reported	4 Variables reported	3 Variables reported	2 Variables reported	Only 1 Variable reported
1.	Deccan Chronicle n=41	2.4	14.6	26.8	14.6	9.7	12.2	19.5
2.	The Hindu n=24	0	16.6	4.2	8.3	8.3	8.3	54.2
3.	Times of India n=24	4.2	37.5	29.2	0	4.2	4.2	20.8
	Sub total- 1 n=89	2.2	21.3	21.3	8.9	7.8	8.9	29.2
4.	Eenadu n=42	0	7.1	21.4	7.1	7.1	14.3	42.8
5.	Saakshi n=12	8.3	25.0	8.3	8.3	0	16.7	33.3
6.	Andhra Jyothi n=71	0	5.6	15.5	7.0	16.9	16.9	38.0
	Sub total-2 n=125	0.8	8.0	16.8	7.2	12.0	16.0	39.2
	Total N=214	1.4	13.6	18.7	8.0	10.3	13.0	35.0

nutrition research. Particularly, in Telugu newspapers not a single report has quoted limitations of the respective research/ study. Only 15.7% of the English reports contain limitations of the respective research/ study. As per the scale constructed for the quantitative assessment of reports on nutrition research, vernacular dailies are lagging behind in comprehensive content, compared to the English newspapers.

In all the three English dailies, 44.9% reports on nutrition research mentioned the sample size, whereas, in Telugu dailies only 30.4% reports contain this variable. Similarly, in all the three English newspapers 47.1% reports described the study population; 52.8% gave description of research variables and 57.3% described study design and analysis, whereas, in vernacular dailies only 31.2% reports stated about the study population; 39.2% mentioned about the research variables and 48.8% revealed study design and analysis.

This study clearly indicates that, no newspaper gave page-one priority to nutrition related topics. During the six-month period, only two research reports, one in Deccan Chronicle (English) and another in Saakshi (Telugu) appeared on page one of the newspaper.

This is the only common factor observed in both the language newspapers. It was observed that, English newspapers mentioned more number of variables, in each of the report compared to Telugu dailies (Table-2). In all the three English dailies, almost a quarter of reports (23.5) consists more than 6 variables in each of the report, whereas it was only 1.6% in all the three Telugu dailies put together. Reports containing with only one variable of nutrition research is more (39.2) in Telugu dailies, compared to (29.2) English dailies.

Of all the reports which appeared in Telugu dailies, 67% of them did not consist more than three variables of the nutrition research, whereas in English dailies 61.5% of reports consists more than three variables. This clearly indicates that, vernacular language newspapers were providing readers with only tips of the nutrition research, but English dailies were publishing these reports with more details. One of the significant finding of this study is that, five reports (one in Saakshi and four in Andhra Jyothi) on nutrition research appeared in regional dailies exaggerated the findings in headlines of the respective reports, whereas only one report with exaggerated headline was found in an English daily (Deccan Chronicle).

In Deccan Chronicle a headline reads “ENERGY DRINKS CAN KILL, WARNS STUDY”. But, it gives details of adverse effects of energy drinks on only such children who suffer with diabetes, seizures, cardiac abnormalities, or mood and behaviour disorders. The original study specifically mentions that, kids suffering with the above mentioned ailments may become hyperactive if they are given over dose of energy drinks. The original study remains silent on the effect of energy drinks on normal kids. Moreover, it is the study conducted only on the children who are with the above mentioned ailments. But, the headline of this report terms energy drinks as killer drinks.

Similar distortion of headlines was observed in few reports of Telugu dailies. Andhra Jyothi, which has published highest number of nutrition research reports during the study period also happens to be topper in over-emphasize of headlines. Reports titled “*Tiffin maanesthe… Gunde Jabbu Khayam!*” (Cardiac ailments Guaranteed… if you skip breakfast); *“Meegada baaga thinte… Moothrasaya cancer guarantee”* (Cancer to Urinary bladder is guaranteed…with more intake of cheese or milk cream); *“Chocolates thinte pandlu thinnatley..!”* (Eating chocolates is equal to fruits intake); *“Suvaasanalathone kovvu karuguthundhi”* (body fat dissolves by fragrance) appeared as headlines in Andhra Jyothi are not complying with the results of original studies, and even overstating the details of the respective news report. Similarly, one headline on nutrition research, titled *“Madhuram Kaadhu Visha bharitham”* (It's not sweet, but full of Poison) appeared in Saakshi exaggerates the news report.

Headlines of some of the news reports have missed the real messages and highlighted only to make it hot news. News headlines tend to focus on partial study results. With medicine being one of the most elusive, mysterious, and misunderstood fields in society, the press often has to unearth the answers to the questions many citizens have. The medical field is constantly pressured to find the next breakthrough and the next lifesaver, but when the slightest information is discovered, the news is everywhere, no matter how accurate the findings may be. Mass media reports are often incomplete. This type of reporting may misinform the lay public and may lead to questions about the applicability of the results to individual patients.

CONCLUSIONS

- Sample characteristics, study methodology, and study limitations were not routinely reported in the six newspapers of the present study.
- These components of research were critical for accurate and meaningful interpretation of nutrition-related research.
- With emphasis on short, "newsworthy" pieces, the media often only reported the results of single studies, and many stories were chosen simply because the results run contrary to current health recommendations.
- The study further stresses the need for synergetic efforts between journalists covering health topics and experts in the field of diet and nutrition, to avoid giving inaccurate information to the readers.

VII FOOD AND DRUG TOXICOLOGY RESEARCH CENTRE

1 CREATION OF DEMAND FOR MILLET FOODS THROUGH PCS VALUE CHAIN

Sorghum is one of the most drought tolerant cereal crops currently under cultivation. Sorghum or jowar can be used to prepare delicious recipes from the flour or rava in combination of other ingredients. Sorghum has abundant fibre both soluble and insoluble. This leads to slow release of glucose in the gastric lumen when consumed; thereby it helps in diabetes and obesity. It has macro and micro nutrients more or less similar to other popular grains. The quality of proteins is poor as compared to other cereal grains. However, some recent cultivars are superior to previously known varieties.

This project 'Creation of Demand for Millet Foods Through PCS value – chain' is aimed at commercializing several products of sorghum for which research was carried out by 4 institutes, viz., Directorate of Sorghum Research, National Institute of Nutrition, Collage of Home Science (ANGRAU) and Agri Business Division of ITC.

At NIN the following studies were undertaken.

I) Nutritional composition of sorghum products
ii) Organoleptic properties of cooked recipes.
iii) Feeding of sorghum foods among school children for 9 months
iv) Controlling blood glucose by replacing 50% of cereals in type 2 Diabetics for 2 months
v) Glycemic Index and Load of recipes based on sorghum
vi) Determining protein digestibility corrected amino acid score (PDCAAS) of sorghum

METHODOLOGY

I) The proximate principles and the micronutrients were estimated using the standard methodology for the products viz sorghum flour, mixed cereal flour, medium grits, sorghum flakes, sorghum pasta and sorghum biscuits.

ii) Fifteen semi-trained evaluators participated in evaluating the recipes along with recipes prepared from rice or wheat. The responses were recorded in 5 point Hedonic scale.

iii) 200 children of residential hostels aged between 9.0 - 12.9 years were enrolled along with 200 controls. Breakfast and lunch food items were replaced with sorghum foods for 9 months.

iv) Enrolled 150 type 2 DM cases and half of their daily cereal intake was replaced with sorghum rotis given during lunch time for two months. HbA1c before and after were measured by chromatographic – Spectrophotometric Ion exchange method.

v) To ten healthy volunteers, seven sorghum based foods and comparable traditional foods were administered at 50g of available carbohydrates (as tested in the raw state) and capillary blood glucose was estimated by glucometer at -5,0,15,30,45,60,90 and 120 min. The average value was taken to calculate the incremental area under curve. Mean serving size from five regions (standard food outlets) was used to determine glycemic load (GL).

vi) Six weanling rats received control stock diet with casein as protein, six received 8% sorghum protein diet (flour) in place of casein and in the last group 6 animals received protein free diet.

Animals were housed in individual cages for 15 days and daily consumption of diet was recorded. In the last 4 days, they were housed in metabolic cages and daily feces was collected in separate containers. Total feces from each animal was lyophilized, weighed and ground. Protein was estimated in the feces by micro Kjheldahl method.

RESULTS

The nutritive values of the sorghum based recipes along with utritive values are as shown in the table

S.No.	Name of the sample	Jowar flour (217.1)	Jowar pasta (217.2)	Jowar flakes (217.3)	Jowar rawa (217.4)	Jowar soya blend (217.5)	Jowar biscuits (217.6)
A. Proximate Composition (g/100g)							
1	Moisture	8.25	8.68	10.55	8.72	7.89	2.76
2	Total Ash	1.33	0.69	0.70	0.90	1.64	2.21
3	Protein	8.76	9.50	7.23	9.79	11.92	6.70
4	Fat	3.24	1.22	1.79	2.87	2.62	23.73
5	Total Dietary fibre	9.69	5.56	5.97	9.23	12.71	5.27
6	Insoluble Dietary fibre	8.1	4.82	5.43	7.92	9.77	3.54
7	Soluble Dietary fibre	1.59	0.74	0.54	1.31	2.94	1.73
8	Carbohydrates	68.73	74.35	73.76	68.49	63.22	59.34
	Energy	342	348	341	342	330	481
B. Minerals (mg/100g)							
1	Calcium	15.42	16.99	10.94	8.81	25.41	68.83
2	Magnesium	140.42	54.32	68.87	79.61	62.90	92.23
3	Copper	0.15	0.43	0.10	0.18	0.22	0.25
4	Manganese	1.01	0.66	0.53	0.91	1.44	0.57
5	Iron	3.52	2.90	3.44	1.84	3.03	2.02
6	Zinc	1.69	0.98	0.88	0.96	1.06	1.69
7	Phosphorous	170.02	110.27	110.02	150.22	85.14	107.66
8	Molybdenum	ND	ND	ND	ND	ND	ND
C. Vitamins (mg/100g)							
1	Thiamine	0.32	0.19	0.18	0.23	0.45	0.23
2	Niacin	1.92	1.15	1.28	1.58	1.93	1.90
3	Riboflavin	0.13	0.11	0.11	0.12	0.15	0.17
4	Total carotenoids	ND	ND	ND	ND	ND	ND
5	Beta-carotene	ND	ND	ND	ND	ND	ND
6	Total folic acid	958.03	732.67	657.8	1283.17	1569.36	547.01
7	Vitamin C	ND	ND	ND	ND	ND	ND

Folic acid in µg Energy in Kcal

ii) All foods tested were found to be acceptable excepting cake based on jowar whose acceptability was significantly lower than control cake based on maida ($p<0.05$). 'Rava Kesari', 'punugulu' and 'chegodi' made with sorghum were significantly better than traditionally prepared foods ($p< 0.05$).

iii) Feeding sorghum foods to school children for 9 months has resulted in better growth and nutritional status. Haemoglobin, S.iron, S.B12 and S.retinol binding protein were significantly higher in sorghum fed children than in control children ($p <0.02$). The other nutritures were similar in both the groups.

iv) Control of blood glucose by replacing 50% of cereals with sorghum rotis among type 2 diabetes subjects for 2 months resulted in decrease of HbA1c (7.87 ± 0.10 , 7.35 ± 0.075; $p<0.01$).

v) Glycemic Index and glycemic load of recipes based on sorghum is given below,

Foods	AC® per 100 gms of food(dry)	Cooked food containing 50gms of AC® (gms)	Glycemic Index	Serving size	Glycemic Load
Rotis	73	115	74	119	38
Multi grain Rotis	99	89	72	151	61
Fine Rava upma	85	313	63	252	25
Coarse rava upma	85	386	60	232	18
Flakes upma	74	129	48	330	53
Pasta*	72	219	49	335	37
Biscuits	85	85	54	75	24

Control	AC® per 100 gms of food(dry)	Cooked food containing 50gms of AC® (gms)	Glycemic Index	Serving size	Glycemic Load
Wheat roti	80	140	65	151	35
Wheat Fine rava upma	68	200	71	252	45
Wheat coarse rava upma	74	255	62	232	28
Rice flakes upma	70	109	88	285	115
Pasta*	66	136	72	335	89
Biscuits	69	69	57	75	31

®AC = Available Carbohydrate; * leftover cooked water is also included

vii) The results of animal experimentation to arrive at the PDCAAS of sorghum proteins are

True protein digestibility of sorghum	89.9%
True protein digestibility of casein	96.7%
PDCAAS% of sorghum proteins	38.4%
PDCAAS % of casein	90.0%

CONCLUSIONS

Higher amount of fibre was present in flour and grits. This grain and its processed products can be made use of in preparing traditional recipes and such foods are organoleptically on par with foods prepared from wheat or rice. They were readily accepted by children and were consumed daily over months and those children could maintain their normal nutritional status. Its regular consumption can help in better control of blood glucose in diabetics. The glycemic index of some products made of sorghum was lower than control foods and the GL of sorghum medium grits is ideal for diabetics. However the protein quality is poor when compared to rice or wheat but the recent cultivars are better than those which were used a decade ago.

2 VALUE CHAIN ON COMMERCIALIZATION OF MAIZE PRODUCTS

India contributes 2.5% of the global maize production and Karnataka and Andhra Pradesh are the corn belts from South. The human consumption is less than 10% of its produce. National Agricultural Innovative Project, a division of ICAR has initiated a project to develop innovative products of maize for commercialization which will generate income for farmers. NIN is a part of the consortium to carry out research on the safety and acceptability. Some maize based products were identified, prepared and studied for its beneficial effects such as controlling of blood sugar in type 2 diabetes mellitus. The following are the aims:

I) To assess the nutritional composition of maize and its products

ii) To study the role of maize in the control of blood glucose among diabetic subjects

iii) To estimate the glycemic index (GI) and load of maize products in healthy volunteers

METHODOLOGY

I) Nutritional composition of maize and its products was done and proximate principles and the micronutrients were estimated using standard methodology (AOAC, 18th edition, 2005).

ii) A community based study was carried out to assess the beneficial effect of maize products in type 2 Diabetes mellitus. HbA1c was measured by chromatographic - Spectrophotometric Ion exchange method both before and after the consumption of maize products.

iii) About 50 g of glucose was administered to ten healthy volunteers as reference food and IAUC was determined in duplicate. Later, test and control cooked recipe foods were administered at 50 g available carbohydrate (as tested in the raw state) using seven foods belonging to two varieties of maize. The capillary blood glucose was estimated by glucometer at -5, 0, 15, 30, 45, 60, 90 and 120 min. The average value was taken to arrive at incremental area under curve. Mean serving size from five regions (standard food outlets) was used to determine glycemic load (GL).

RESULTS

I) Nutritional composition of two varieties of maize products, Nityashree and Quality Protein Maize (QPM) were analysed and the results are given in the tables 1 and 2.

Table 1. Nutrient composition of Nityashree Maize Recipes

All values as per 100gms of cooked portion

RECIPE / NUTRIENTS	Flour	Roti	Upma	Vermicelli	Noodles	Crunches
Carbohydrates (g)	87.49	67.30	70.09	79.5	77.98	49.90
Proteins (g)	6.48	5.20	11.18	10.72	12.74	9.80
Fats (g)	4.44	3.20	14.07	5.24	3.76	33.90
Soluble fibers (g)	1.3	1.20	5.52	9.38	0.18	0.90
Insoluble fibers (g)	12.2	10.80	24.46	32.04	21.67	11.70
Thiamine (mg)	5.35	2.50	1.32	<0.02	11.38	10.00
Riboflavin (mg)	3.87	0.10	0.05	0.07	<0.02	0.50
Niacin (mg)	25.03	24.60	1.32	1.73	<0.02	1.80
Pyridoxine (mg)	50.97	34.40	0.53	0.50	71.99	9.30
Folic acid (mg)	14.11	6.10	0.16	0.09	<0.02	11.00
Ascorbic acid (mg)	2.39	2.10	11.3	8.52	16.54	5.20
Beta carotene (ug)	2799	1090	7917	2624	1038	15.50
Calcium (mg)	9.67	14.70	80.75	55.27	81.95	58.90
Iron (mg)	2.05	1.80	1.32	5.88	10.9	3.50
Copper (mg)	0.16	0.12	0.05	4.42	3.15	0.50
Magnesium (mg)	9.90	8.60	71.02	55.27	45.41	43.80
Chromium(mg)	0.34	1.0	<0.03	0.02	0.09	4.0
Zinc (mg)	2.05	1.90	1.05	7.65	22.77	1.80

Table 2. Nutrient composition of Maize QPM Recipes.

All values as per 100gms of cooked portion

RECIPE / NUTRIENTS	Flour	ROTI	UPMA	Vermicelli	Noodles	Crunches
Carbohydrates (g)	86.2	88.2	70.22	86.2	84.04	66.29
Proteins (g)	7.42	7.21	9.37	8.4	6.65	6.3
Fats (g)	4.59	3.09	13.38	1.56	4.28	25.21
Soluble Fibre (g)	1.0	2.27	5.98	7.23	3.76	2.12
Insoluble Fibre (g)	12.9	18.12	24.42	18.22	21.87	18.7
Thiamine (mg)	0.02	0.07	0.59	<0.03	1.72	<0.01
Riboflavin (mg)	0.06	0.07	0.12	0.10	<0.01	<0.01
Niacin (mg)	0.10	0.14	1.47	1.06	1.72	<0.01
Pyridoxine (mg)	0.02	0.42	0.21	0.74	0.07	<0.01
Folic acid (mg)	0.10	0.10	0.06	0.10	<0.01	<0.01
Ascorbic acid (mg)	<0.1	<0.1	2.36	7.08	9.76	0.4
Beta carotene (µg)	2740	2195	1380	2209	1298	1114
Calcium (mg)	114	111.7	75.02	108.8	117.7	76.9
Iron (mg)	2.54	1.98	2.35	4.36	4.86	5.8
Copper (mg)	0.01	0.28	0.03	0.42	0.87	0.39
Magnesium (mg)	105.7	101.9	95.0	89.5	38.2	46.1
Chromium (mg)	<0.01	<0.01	0.03	0.08	0.11	0.03
Zinc (mg)	2.2	2.41	1.77	3.12	2.7	1.28

ii) For the study on Type 2 diabetes subjects, 31 subjects were enrolled. At the end of two months after consumption of sorghum rotis at 50% of their daily cereal intake given around noon, the subjects were again interviewed for their status and examined.

The study was completed in 30 subjects. The blood HbA1c got decreased from 8.1±0.289 % to 7.47±0.195 %; $p<0.05$.

iii) The GI and GL as determined in both varieties are depicted in table 3.

Table 3. Glycemic load of maize based products

S.no	Recipe	Glycemic index	Serving size	Glycemic load
Nityashree Maize(2049)				
01	Roti	93	151	50
02	Fine rava upma	94	252	41
03	Coarse rava upma	80	232	25
04	Noodles	76	502	42
05	Vermicelli	77	220	39
QPM(Quality Protein Maize)				
01	Roti	86	151	66
02	Fine rava upma	79	252	28
03	Coarse rava upma	78	232	30
04	Noodles	66	502	30
05	Vermicelli	72	220	30
Control foods				
1	Wheat roti	80	151	35
2	Fine rava upma	68	252	45
3	Coarse rava upma	74	232	28
4	Noodles	85	502	51
5	Vermicelli	67	220	39

CONCLUSIONS

- QPM Maize had better protein quality than Nityashree. Maize had greater amounts of carbohydrates than most other grains with more fibre than other cereal grains.The grain also contributes to β carotene in traces.
- The prolonged use of maize among type 2 diabetic subjects showed significant decrease in HbA1c levels, perhaps due to fiber content in the grain.
- They were not low in GI and GL though through regular consumption the GL may be lowered.

3 MICRONUTRIENT PROFILE OF POPULATION RESIDING IN FLUORIDE ENDEMIC AREAS

Fluorosis is a disease caused due to chronic exposure to high fluoride and is a major public health problem when fluoride level in drinking water exceeds 1-1.5ppm. An estimated 62 million people in 22 states in India are affected with dental and skeletal fluorosis. Fluoride when ingested through food, water or by other means enters the gastrointestinal tract where it reacts with HCl and replaces Cl with F forming hydrogen fluoride (HF), which is corrosive in action. Hydrogen fluoride acts on intestinal mucosa and destroys intestinal microvilli resulting into non-absorption of nutrients in the gastro-intestinal tract that may alter the nutritional status of an individual resulting in malnutrition and enhances fluoride risk in fluorotic area. Fluorosis is aggravated by malnutrition. Even the water composition has been shown to affect the incidence and severity of fluorosis. It is reported that the drinking water in fluoride endemic area had significantly lower Ca, Mg and Cu content as compared to control villages. A similar observation was reported in the villages where genuvalgum was prevalent. Copper deficiency results in decreased bone collagen synthesis and increased bone mineralization. The bio availability of fluoride and severity of fluorosis is amplified in deficiency of calcium, zinc, copper, magnesium vitamins (vitamin D) and / or amino acids. On the other hand it is also reported that fluorosis causes micronutrient deficiency in animal models as well as humans. In a study, subjects with fluorosis were shown to have lower serum micro-nutrient mineral levels (Cu, Zn, Mn and Mg) than healthy subjects. This deficiency has been implicated in the pathogenesis of fluorosis. Further, it was reported that, among sheep exposed to fluoride in the natural conditions revealed significant differences in the blood levels of ß-carotene, vitamins A and C as compared to unexposed sheep. Whether the fluoride toxicity creates micronutrient deficiency or micronutrient deficiency is exaggerating fluoride toxicity is not clear. If micronutrient deficiency is the cause, there is a possibility that appropriate supplementation of micronutrients (Vitamins & minerals) in fluorotic subjects can overcome fluoride toxicity and its complications. However, there is no such controlled study that establishes the role of micronutrients in the pathogenesis of fluorosis and its complications. Hence, the present study has been planned to know the role of micronutrients in the development of fluorosis in humans.

Aim: To evaluate the association of micronutrients (minerals and vitamins) with fluorosis.

OBJECTIVES

1. To study micronutrient status (Vitamins A, D, E, B1, B2, B12, and folic acid), trace elements like Fe, I, Ca, Mg, Zn, Mn, Cu, Se, and Mo) in blood in sub samples of population.
2. To study the nutritional status of population by anthropometry.
3. To estimate fluoride level in drinking water and in urine.
4. To study diet and nutrient intake of study population.

METHODOLOGY

Design

It was community based cross sectional study. The study was approved by Institute's ethics committee. All individuals (Socio economically matched**),** aged between 8 to15 (children) and >55 years (adults) (age and sex matched) were selected from the randomly selected fluorotic (F=8.0 ppm, Venkapally) and non fluorotic (F≤1.0 ppm, Rampally) villages. A total of 244 subjects were selected from fluorotic (182) and non-fluorotic villages (62). The ethics committee of Institute approved the study protocol. Informed consent was obtained from the subjects.

Clinical examination

The signs and symptoms of dental and skeletal fluorosis were examined by a clinician.

Dietary intake

It was calculated using 24-hr recall method. The intake of different foods was converted to nutrients per consumption unit using food consumption table.

X-ray

All the selected subjects from affected and control villages were brought to Kamineni Hospital, Hyderabad and their posteroanterior radiographs of chest, anterioposterior and lateral view of the lumbar spine, anterioposterior views of elbows, forearms, wrists and knees were obtained.

Dual energy X-ray absorptiometry (DXA)

Bone mineral density (BMD) (g/cm^2) and bone mineral content (BMC) were measured at anterioposterior (AP) lumbar spine using DXA (Hologic 4,500 W, Waltham, Massachusetts) at three sites (L1-L4), the hip including the femoral neck (FN), forearm and the whole body (WB). All the scans and analysis were carried out according to the manufacturer's instructions by a trained technician, as per the guidelines spelt out by the manufacturer.

COLLECTION OF BIOLOGICAL SAMPLE

Blood

About five ml of blood (fasting) was collected from 20% of randomly selected subjects and transported to the laboratory immediately in ice. Serum was separated and stored at -85^{0}C until further analysis.

Urine collection

Early morning spot urine samples was collected in a fresh plastic bottle containing 2-3 drops of toluene to avoid microbial growth. Collected urine samples were brought to the lab in cold condition and kept at cold condition (4°C) till further use.

BIOCHEMICAL ESTIMATIONS

Urine

Fluoride in water, plasma and urine was estimated using Orion ion specific electrode (EA940, Boston, MA, USA).

Serum

About 25 hydroxy vitamin D, 1, 25 dihydroxy vitamin D, and intact parathyroid hormone were analyzed using RIA Kits supplied by Diasorin- USA. Total and bone specific alkaline phosphatise (ALP), tartarate resistant acid phosphatase (TRAP) was analyzed by Klaus Walter and Moss method. Serum T_3 and T4 were analyzed using RIA kits and thyroid stimulating hormone was analysed using IRMA kit supplied from BRIT (DAE-INDIA).

Elemental analysis

Calcium, magnesium, zinc, selenium and copper were analyzed using atomic absorption spectrophotometer (Perkin Elmer, Lambda 1 UV/VIS).

RESULTS

Clinical: The signs and symptoms of fluorosis like dental mottling of different grade is shown in figure 1. Body pain, bomboospine, neck rigidity, kyphosis, genu valgum, genu varum and exostosis (Figure 1) were significantly higher in affected village as compared to control village.

Biochemical

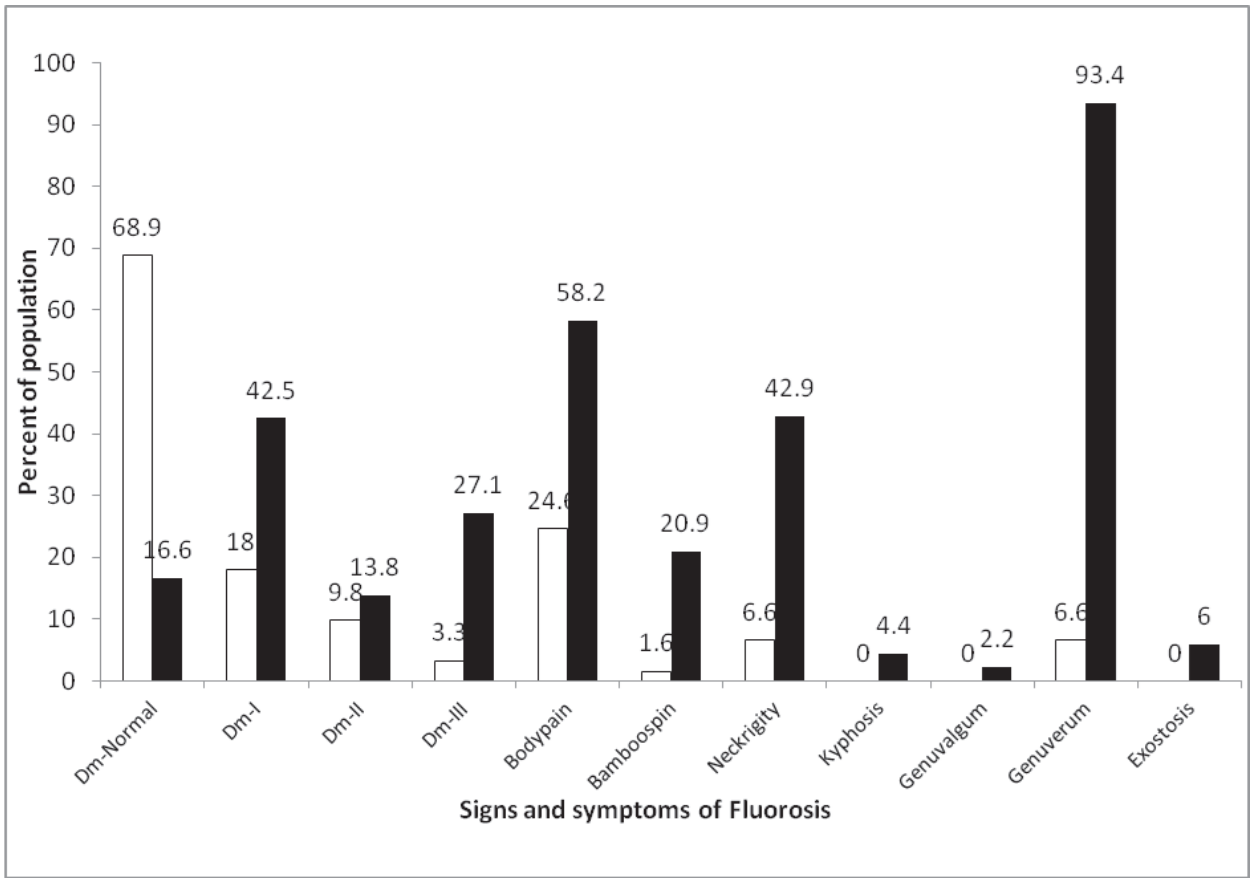
1. Urinary fluoride in affected and non affected from affected village was significantly higher as compared to control village (Table-1).

Table 1. Urinary and serum fluoride (Mean± SD) in control and affected village

Age group	Urinary fluoride (ppm)			Serum fluoride (ppm)		
	Control	Non affected	Affected	Control	Non affected	Affected
15±24	2.27±1.03	8.7±2.311[a]	8.5±4.2[a]	0.09±0.003	0.07±0.038	0.13±0.065[b]
25±34	2.47±1.65	6.51±3.47[a]	9.52±1.05[a]	0.09±0.006	0.22±0.189	0.11±0.061
35±44	3.0±3.42	7.0±4.89	13.9±10.74[a]	0.1±0.014	0.17±0.061[a]	0.13±0.059
45±54	3.1±1.49	8.0±6.46	12.3±5.02[a]	0.09±0.009	0.19±0.130[a]	0.11±0.063
>55	2.4±1.29	9.8±4.26[a]	10.5±2.52[a]	0.11±0.021	0.102±0.089	0.21±0.099[ab]

a= comparison with control group, b= comparison with non effected group
c= comparison with effected group

Figure 1. Signs and symptoms of fluorosis in affected and control subjects



2. Serum fluoride was significantly higher in affected village (age 15-24) as compared to non affected as well as in age groups >55 as compared to control and affected groups. Serum fluoride in non affected group (age 35-54 years) was significantly higher as compared to control.

3. Serum 25 (OH) vitamin D, PTH, total acid phosphatase and TRAP were significantly lower in all the age groups in affected village as compared to control village. Total ALP, Bone specific ALP was significantly higher in affected village as compared to control village.
4. Magnesium, selenium and zinc were significantly higher in affected village as compared to control village in all the age group whereas there was no significant difference in copper and strontium in both groups.
5. The thyroid function test was assessed by analyzing T3, T4 and TSH in affected and control village. T3 was significantly higher in affected village than control village whereas T4 was significantly lower in affected as well as in non-affected subject of affected village than control village subjects.

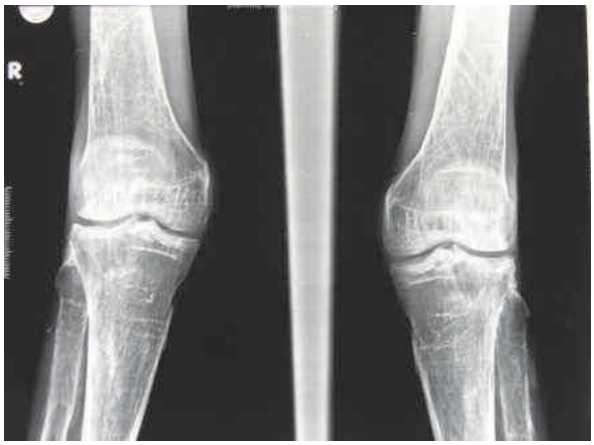
Haematology: All haematological parameters are comparable except haemoglobin which was significantly lower in affected village as compared to control village.

DXA: The area, BMC and BMD was checked and analyzed in areas like hip, whole body spine, neck, trochanter, L1 to L4 ribs T spine, L spine, L leg. Out of these areas DXA showed neck, trochanter, R-ribs, T-spine, L-spine and L-leg were significantly higher as compared to control. Whereas L1 to L3 (area, BMC and BMD) were significantly higher in affected group as compared to control as well as non affected groups of affected village. However, there was no significant difference in hip whole body and spine in area, BMC and BMD.

Dietary Intake: The intake of fruits and sugar was lower in affected village as compared to control village. Whereas, milk and milk products intake was higher in affected village as compared to control. The calcium and phosphorous was higher in affected village as compared to control village. Folic acid, free folic acid was higher in affected village as compared to control village. However, there was no significant difference in all nutrients intake among the groups.

X-ray: Genu varum, severe osteoporosis, narrowing of joint space osteoarthritis changes in both the knees, increased bone density and exclusive osteophyte formation in lumbar spine with lumbar canal sclerosis were observed. However, any neurological involvement was not seen. Bilateral genu varum severe degenerative changes joint space narrowed (Figure 2). Increased bone density with severe degenerative changes in ankle joint with joint space narrowing and osteophyte formation and severe lumbar canal sclerosis was also observed.

Figure 2. X-Ray photograph of lower limbs of fluorotic patient with trace lines showing calcium deficiency



CONCLUSION

The effect of high fluoride water was less than observed elsewhere at this concentration, apparently owing to better nutritional status in the fluorotic village.

4 ASSESSMENT OF PESTICIDE EXPOSURE AND VARIOUS CANCERS AMONG AGRICULTURAL FARMING COMMUNITY GUNTUR DISTRICT

Organochlorine pesticides have received worldwide attention in the last few decades since studies have shown an accumulation of these chemicals in the environment and in human tissues. The biological monitoring of persistent and toxic pesticides is very important in countries like India. Several studies conducted in India have shown organochlorine pesticide residues in human blood samples. The chlorinated compounds are fat soluble and can be easily measured in serum. Literature indicates presence of organochlorine pesticide residue levels in human blood, but the information on presence of organophosphorous pesticide residues is meager.

Majority of the farmers in Guntur district use organophosphate and synthetic pyrethroid pesticides for the control of pests on commercial crops like chilly and cotton. Many of the cases that are reported to Guntur Govt. General Hospital, and Bommidala Cancer Care Research Institute were found to be Lymphoma, Leukemia and Breast cancer. Most of these were seen to affect farming community. Review of literature indicated that there was an association between the risk of breast, lymphoma and Leukemia cancers in the agricultural farming community.

AIMS AND OBJECTIVES

I) To study the extent of exposure to pesticides among the cancer Patients admitted in Guntur Govt.Hospital/ Bommidala Cancer Care Research Institute in Guntur District.

ii) To study the association between the pesticide exposure and cancer development in individuals visiting selected hospitals of Guntur district.

iii) To assess the socio-demographic profile of subjects and agricultural practices which include types of pesticides used, quantity of pesticides used, duration of exposure etc. clinical and pathological assessment of cancers with respect to staging and typing.

iv) To analyse the AchE in the subjects (cancer patients) along with the selected pesticide residues/metabolites in the plasma of the subjects.

Work done during the year

METHODOLOGY

Study Design: Both males and females who are visiting/admitted and diagnosed for various types of cancers from GGH and Bommidala Cancer Care Research Institute and whose age group ranged from 18-70 years were included for the present study. 8-10mL of the venous blood samples from them (670) was collected and used for analysis for measuring pesticide residues as well as the selected biochemical parameters namely viz., RBC AChe, plasma Butyrylcholinesterase (BuchE) etc. Pesticide residue analysis was done using GC/MS and LC/MS/MS. The subjects selected in the present study were mostly illiterates.

The farmers were seen to be using a variety of pesticides belonging to different groups namely organochlorines/ organophosphorous/ synthetic pyrethroids/ Carbamates and others for cultivating the agricultural land holding of about 4 acres of other farmers (Table 1). The subjects were using 500mL to 2.5 litres of different types of pesticides per acre.

The subjects in the present study were agricultural labourers who were engaged in the various pesticide spraying activities, mixing up of formulations etc. and other activities such as ploughing, sowing, thrashing, harvesting etc. While responding to the interview schedules, these subjects had mentioned about the effects of exposure to pesticides including red eyes (96%) followed by burning

sensation in eyes (92%) and with headache (64%), itching of the skin (51%), lacrimation of the eyes (20%) and tingling sensation (11%).

The subjects from selected hospitals of Guntur District were found to be suffering from certain cancers affecting Lymphoid tissue, Genital tract, Skin and soft tissue, Bones, Urogenital tract, Oral cavity, CNS, Respiratory tract, Endocrine, Breast and Gastro-intestinal tract. ANOVA was used to assess the difference in RBC AchE inhibition activity and plasma BchE activity, with respect to duration of cancer in years and grades of cancers.

It was found that the inhibition of RBC AchE activity (normal range-1990 U/L) was found to be more for ≥5 years (1592) followed by 4years (1661) and ≤3years (1673) (Table 2).

It was also observed that inhibition of the same was more among the subjects who were found to have diagnosed for grade-4 cancer as compared with grades 1, 2 and 3. However, the inhibition of plasma Butyrylcholinesterase (BuchE) activity was also found to be showing the similar trend as that of RBC AchE activity (Table 3).

For assessing the difference in duration of exposure (in years) vs RBC AchE activity and plasma BchE among the subjects 't'-test was performed (Table 4).

RESULTS

Of the 670 subjects analysed for the pesticides residues from both the hospitals, 89 subjects showed p,p' DDE at a level 6.041± 2.659 ppb to 18.05 ± 10.091ppb. Of the various pesticide residues detected among the subjects o,p' DDE was detected at a range of 65.176±19.8 in 4 subjects diagnosed for oral cavity

Table 1

S.No	Variable Name	Group	n	Valid %
1	Quantity of pesticide sprayed/day (Ltrs)	=1	277	96.9
		2, 5	9	3.1
2	Quantity of pesticides used (ml/acre)	=500	137	43.8
		>500	176	56.2
3	Other Works carried out in addition to spraying activities	Ploughing	464	69.2
		Cutting	602	89.8
		Watering	505	75.4
		Thrashing	540	80.6

Table 2

Choline esterase activity Vs cancer duration				
S. No	Cancer Duration	n	Choline Esterase activity	
			*RBC-AchE U/L (Mean ± SE)	**Plasma-BchE U/L (Mean ± SE)
1	=3	116	1673 ± 53	3312 ± 93
2	4	347	1661 ± 32	3381 ± 56
3	=5	206	1592 ± 39	3445 ± 68

*p=0.331 (NS); **p=0.521 (NS)

Table 3

S. No	Cancer grade	n	Choline Esterase activity	
			*RBC-AchE U/L (Mean ± SE)	**Plasma-BchE U/L (Mean ± SE)
1	1	193	1674 ± 44	3488 ± 73
2	2	242	1632 ± 36	3339 ± 66
3	3	62	1681 ± 92	3535 ± 147
4	4	70	1627 ± 70	3134 ± 124

*p=0.847 (NS); **p=0.053 (NS)

Table 4

Duration of exposure in years Vs Choline esterase enzyme activity (Mean - SE)				
S. No	Duration exposure (yrs)	n	Choline Esterase activity U/L (Mean - SE)	
			*RBC-AchE	**Plasma-BchE
1	= 10	79	1625 ± 50	3506 ± 104
2	>10	233	1621 ± 41	3361 ± 74

*p=0.949 (NS); **p=0.301 (NS)

cancer (Table 5).With respect to the analysis done for the other groups of pesticide residues, lamda-cyhalothrin was detected among 667 subjects at a range of 10.8 to 41.7 ppb (Table 6).

With respect to the operational mode of the pesticides among the subjects, it was revealed that they were using both hand pump and power spray (Table 7). However, very few were using one or the other protective devices such as gloves, mask, apron, shoes etc as they do not feel comfortable to use the protective devices while spraying the pesticides onto crops (Table 8).

Table 5 Detected pesticide residues in various cancers

Sl No	Type of Cancer	Organochlorine Pesticide Residues (Conc in ppb) Mean - SE							
		n	p,p DDE	n	p,p DDD	n	p,p DDT	n	o,p DDE
1	Lymphoid tissue	8	9.979 ± 2.196	3	10.338 ± 1.281	1	5.611		
2	Genital tract	32	9.49 ± 1.403	4	5.491 ± 1.581	4	10.438 ± 1.219	1	1.55
3	Skin & soft tissue	3	14.749 ± 2.488	3	6.924 ± 1.642	3	12.926 ± 4.39		
4	Bones	1	3.67						
5	Urogenital tract	2	6.041 ± 2.659			1	5.648		
6	Oral cavity	4	18.05 ± 10.091					2	65.176 ± 19.82
7	CNS	5	10.925 ± 3.814						
8	Respiratory tract	14	12.174 ± 3.068	3	6.56 ± 2.684	3	10.981 ± 4.626	1	5.632
9	Endocrine	2	11.415 ± 9.765						
10	Breast	11	11.303 ± 2.201	1	8.214	1	5.023		
11	Gastro-intestinal	7	11.745 ± 1.508	4	7.684 ± 1.199	2	13.629 ± 8.26		

Table 6

Sl No	Type of Cancer	n	Type of pesticides used Vs cancer type (%-with in cancer type)							
			Synthetic Pyrethroids			Others				
			a-Methrin	L-Cyhalo	Cyper	Spino-sad	Metho-myl	Ace-tamprid	Mix (>1)	Mix (>2)
1	Lymphoid tissue	49		38.8	14.3	8.2		12.2		
2	Genital tract	269		16.4	17.5	3.3	0.7	3		0.7
3	Skin & soft tissue	18		22.2	33.3					
4	Bones	9		33.3	44.4	22.2		11.1		
5	Urogenital tract	16		37.5	37.5	6.3		6.3		
6	Oral cavity	65	1.5	10.8	12.3	10.8	1.5	4.6	1.5	
7	C N S	15		33.3	33.3	13.3		26.7		
8	Respiratory tract	60	1.7	41.7	35	8.3	3.3	6.7	1.7	
9	Endocrine	10		20	20			10		
10	Breast	78		25.6	19.2	5.1		5.1	1.3	
11	Gastro-intestinal	78		23.1	23.1	14.1	1.3	6.4		1.3

Table 7

S. No	Protective devices	n	%
1	Yes	118	17.7
2	Gloves	100	84.7
3	Shoes	2	1.7
4	Mask	37	31.4
5	Apron	10	8.5
6	Any other	3	2.5

Table 8

No	Protective devices	n	%
1	Inconvenient to work with gloves	40	7.3
2	No access to wear apron & shoes	83	15.1
3	Cannot judge whether spraying is proper or not	2	0.4
4	Others	10	1.8

Table 9

S. No	Symbol	n	%
1	Red-Extremely toxic	4	0.7
2	Yellow-highly toxic	1	0.2
3	Blue-Moderately toxic	1	0.2
4	Green-less toxic	1	0.2

The KAP studies data showed that very few subjects were aware about the various toxic symbols mentioned on the pesticide containers which indicate toxicity to humans (Table 9). As regards the precautionary measures that are to be followed while spraying the pesticides, majority of them said they do not read them (Table 10).

With respect to the hygienic practices which are supposed to be followed after spraying the pesticides on to the respective crops, majority of the subjects reported water for washing their hands immediately after spraying (Table 11). While pertaining to the storage of pesticide containers very few stored them in the farm/s after their use/spray (Table 12).

Table 10

S. No	Reading of precautions	n	%
1	Always	2	0.3
2	Rarely	12	1.7
3	Never	653	97.5
	No reading because		
4	Cannot read	554	90.8
5	Never thought of it	42	6.9
6	Other reasons	7	1.1

Table 11

No	Measures	n	%
1	Wash hands with water immediately after use	300	75.6
2	Wash hands with soap immediately after use	38	9.6
3	Take bath immediately after use	28	7
4	Change work clothes immediately/daily	31	7.8

Table 12

No	Storage	n	%
1	Store in the farm	58	17.5
2	Store in the house in a separate room	138	41.6
3	Store in the house along with others	136	41

However, further studies are required by including the cancer subjects who were not exposed to pesticides during their life time and also analysing molecular/ genotoxic parameters may give a lead to get a comprehensive understanding on the various factors responsible for causing cancer among exposed and unexposed subjects before arriving at any definite conclusion.

5 ASSESSMENT OF DIETARY INTAKES OF SELECT CHEMICAL AND PROCESSING- INDUCED CONTAMINANTS IN VARIOUS SOCIO-ECONOMIC GROUPS IN HYDERABAD

The levels of nutrients and contaminants in the studies conducted in India hitherto were calculated and expressed on the basis of raw foods and not on cooked foods. They did not provide the exact dietary intake and exposure levels to the population consuming it, as processing of the foods might lead to changes in their concentrations. The amount of the food consumed by the population also decides the levels of intake and exposure and the extent of potential risk, if any.

The data of the National Nutrition Monitoring Bureau (NNMB), provide information about the amount of conventional foods consumed but the consumption pattern of processed foods in the urban areas is yet not reported. There is a lack of data on different trends in the consumption in various socioeconomic strata of the population. The pilot study shows that the dietary habits of these groups are different. The type and the amount of foods eaten by each group are different from the other. Generally, High Income Group (HIG) shows a high intake of fruits, processed foods, milk and milk products, meat; fish etc., whereas the intake of cereals and pulses along with vegetables is high in Middle Income Group (MIG) and Low Income Group (LIG). The quantity of each commodity also varies from strata to strata. Incidences of eating out are also high in case of HIG which actually leads to high consumption of trans- fatty foods along with high sodium intake. Thus analysis of these groups separately tends to provide a comparative data as to how the consumption of every nutrient and contaminant is varying in each of the strata of the population.

This study intends to detect the concentrations of various nutrients and contaminants in foods after being subjected to common household processing in Hyderabad city and to assess the dietary exposure estimates in various SE groups. The foods to be analyzed were in *"table ready"* form.

AIM

To study the levels of exposures to contaminants through daily diets among the various socioeconomic sections of Hyderabad city and to assess its risk, if any.

OBJECTIVES

- To screen the most commonly consumed conventional and processed foods among various socioeconomic sections of Hyderabad.
- To collect the samples of these most commonly consumed foods from the representative points in the city.
- To detect and quantify the levels of select contaminants (pesticides, heavy metals and trans fatty acids) in the selected foods in table ready form.
- To estimate the intakes of the contaminants among various socioeconomic sections.
- To compare the intakes with the reference standards as Acceptable Daily Intakes (ADIs) and Provisional Tolerable Weekly Intakes (PTWI) and recommended limits.

STUDY DESIGN

A diet survey was conducted with a validated food frequency interview schedule (FFIS) as a tool, among the various socioeconomic sections (SES) (High income group, middle group, low income group, industrial laborers and slum dwellers) of Hyderabad and a total diet food list of most commonly consumed conventional (staple) and processed foods was compiled. The selected

foods were procured from the various markets representing each of the SES. The foods were processed in the laboratory as common Indian house hold level cooking. The processed foods were analyzed for trans fatty acids, pesticide residues and toxic heavy metals (lead and cadmium). The intakes of each of the chemicals were calculated at mean and 95th percentile consumption levels and were compared with their reference standards to check whether they were within the specified limits, and to find out if any difference among the SES level intakes and the intakes do not pose exposure risk to the population of Hyderabad.

SALIENT FINDINGS

- Dietary intakes of conventional and processed foods differed among the SES in Hyderabad.
- Intakes of TFA through selected bakery items and natural sources were less than 1% of energy/day (WHO suggested safe intakes) at mean and highest consumption levels.
- Estimated dietary intakes of lead and cadmium were below the respective provisional tolerable weekly intakes (PTWIs).
- At 95th percentile levels of food consumption, lead intakes exceeded the PTWIs in all SES probably due to contamination of drinking water and higher intakes of cooked rice.
- Drinking water was also found to be contaminated with β HCH above the MRL levels.
- Milk was also found to be contaminated with β HCH above the MRLs.
- The Estimated dietary intakes of all the pesticide residues analyzed were also within their respective Acceptable Daily Intakes (ADIs) at both mean and 95th percentile levels; however those of β HCH were approaching the ADIs at 95th percentile consumption levels.

6 EVALUATION OF HERBAL AND NUTRACEUTICAL PRODUCT FOR ANTI-ATHEROSCLEROTIC ACTIVITY

Atherosclerosis is one of the major causes of death worldwide. Earlier research findings (Annual Report 2010-2011-2012) have demonstrated anti-atherosclerotic activity (in-silico, *in-vitro and in-vivo*) of Poly Herbal Nutraceutical Formulation (PHN). In the current investigation inhibitory foam cell formation mechanism of PHN against CD36 signaling cascading pathway which promote entrapment of lipid laden foam cells within intimal lesions. This process involves inflammation and oxidized LDL (oxLDL) activation, Focal Adhesion Kinase (FAK formation of foam cell due to ROS,) leading to foam cell formation and accumulation of lipids in the walls of arteries.

AIM

To evaluate the anti-atherosclerotic activity of PHN (Poly herbal and nutraceutical) formulation.

OBJECTIVES

- To screen the selected herbals and nutraceuticals extracts for potential anti-oxidant, anti-inflammatory and anti-hyperlipidemicactivity.
- To assess inhibitory effect if any on the “foam cell formation” of selected herbals and nutraceuticals extracts.

- Qualitative characterization of chemical constituents of extracts using various analytical techniques.
- To evaluate anti-atherosclerotic activity of above potential extracts in suitable animal model.

Work done during the year

METHODOLOGY

The following techniques have been utilized.

- *Cell viability assay* IC_{50} concentration of all the test materials was calculated by MTT cell viability assay.
- *Foam cell formation assay* was evaluated by mice macrophage J774A.1 cells which were treated with PHN from (0.001–0.2 mg/mL), and LPS (5 µg/mL) and LDL (50 µg/mL) for 24 h. Lipid accumulation within the cell was stained by Oil-Red O-stained which was extracted in iso-propanol and quantify spectrophotometerically (520 nm) and morphologically observed by Oil-Red O-stained and hematoxylin stains.
- *Western blot analysis* was used to quantify the effect of PHN on CD36, PPAR-γ and FAK proteins in mice macrophage J774A.1 cells treated with LPS and LDL.
- *Inhibition of ROS generation* was assessed by florescent microscopy in LPS treated Raw 264.1 mice macrophage cell.

RESULTS

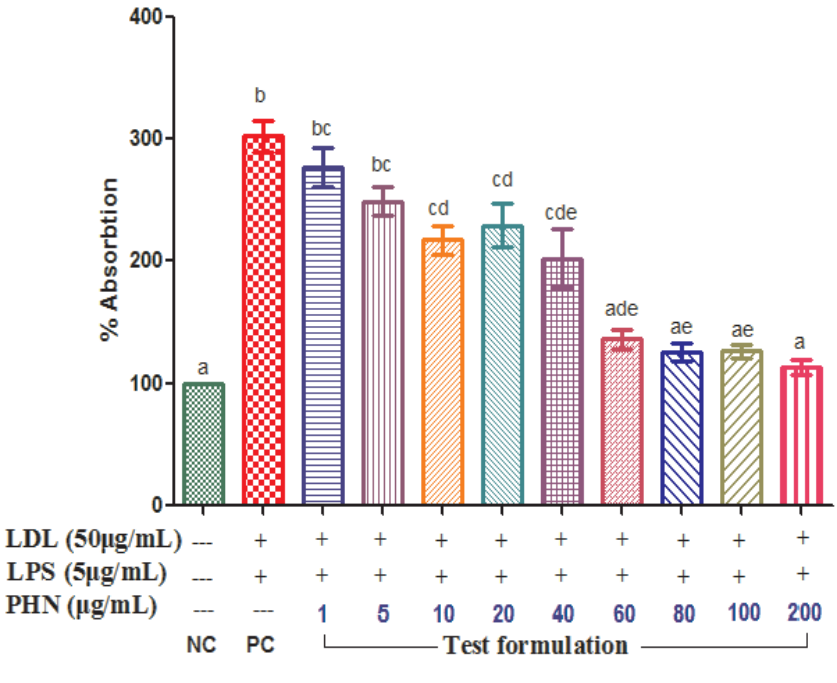
Cell viability MTT assay

- Ic_{50} of PHN was found to be 865µg/mL, which is inversely proportional to the cell viability.

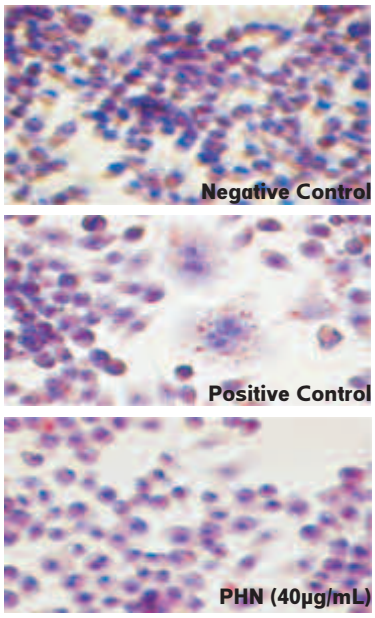
Foam cell formation assay

- The inhibition of foam cell formation by treatment with PHN (1–60µg/mL) was found to be dose dependent (Fig. 1).

Figure 1. Effect of PHN on foam cell formation



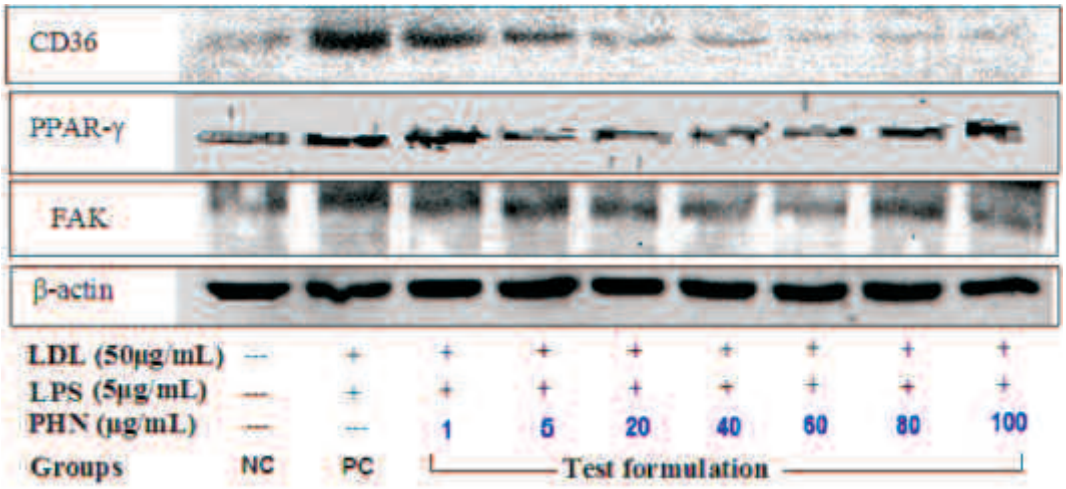
Quantitative analysis by spectrophotometer



Western blot analysis

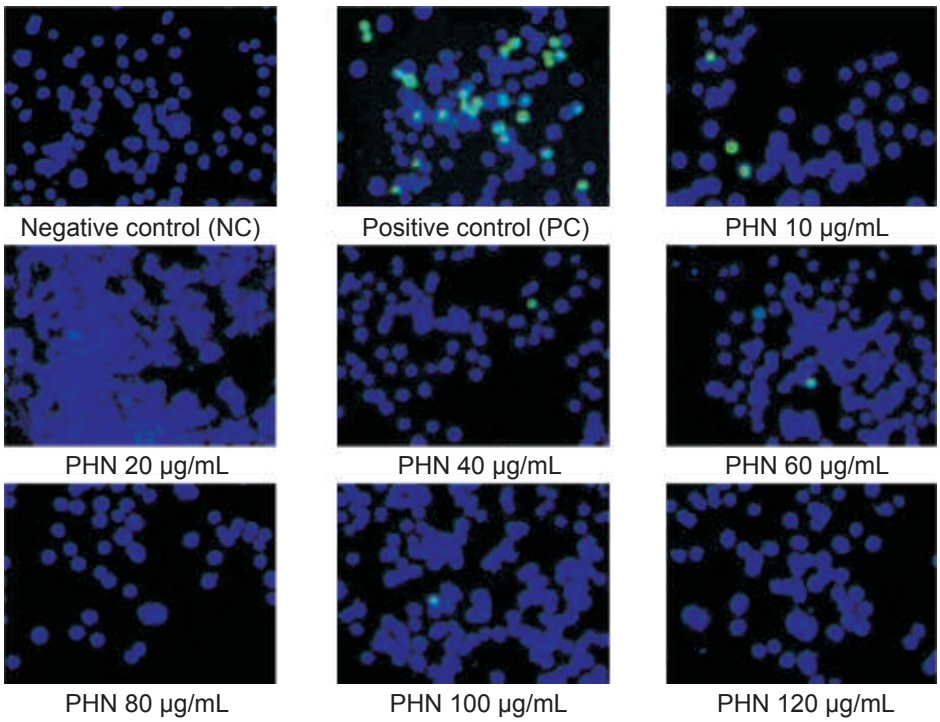
The CD36, PPAR-γ and FAK protein expression levels were found to be decrease significantly with increase in concentration of PHN (1-100 g/mL) (Fig.2).

Figure 2. Effect of PHN on CD36 and PPAR- protein levels in mouse macrophage



ROS generation: LPS induced ROS generation was inhibited by treatment of PHN at concentration 1-120μg/mL in mice microphage cell (Raw 264.1) as shown in Fig.3.

Figure 3. Macrophage exposure to LPS induces generation of ROS



CONCLUSION

Poly herbal and nutraceuticals formulation prevents foam cell formation possibly through attenuation of CD36 scavenger receptor signaling cascade. *In vivo* and *in silico* experiments are in progress.

VIII NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

A SERVICE ACTIVITIES

1. Breeding and supply of animals

During the period a total of 25,964 animals were bred and out of which 20,815 animals were supplied to various organizations and 1884 animals were supplied within the institute. A total of Rs.51, 58,715 (Rupees fifty one lakhs fifty eight thousand seven hundred and fifteen only) has been generated. Details of individual strains of animals bred and supplied are shown in Tables 1&2.

2. Supply of Animal Feed

a. Stock Animal Feed

The stock feed of 73,880 Kgs (Rat and Mouse feed 62,600 Kgs + Guinea pigs and Rabbit feed 11,280 Kgs) was prepared during the period. Out of this, a total of 32,611 Kgs feed (Rat and Mouse feed 26,372 Kgs + Guinea Pigs and Rabbit feed 6,239 Kgs) was supplied to other organizations during the period generating an amount of Rs.36,22,102/- (Rupees thirty six lakhs twenty two thousand one hundred and two only). An additional quantity of 41,269 Kgs of feed (Rat and Mouse feed 36,211 Kgs + Guinea Pigs and Rabbit feed 5,058 Kgs) was also supplied within the institute. The details of the stock feed supplied are shown in Table 3.

b. Experimental Animal Feed

In addition, Centre also prepared custom made experimental animal feed and supplied 242 Kgs this year to 11 institutions on 18 different occasions and generated an amount of Rs. 1,90,318/- (Rupees one lakh ninety thousand three hundred and eighteen only).

3. Blood and Blood Products

During the period, a total of 1300 ml of Blood and blood products have been supplied to 9 different institutions on 24 different occasions and an amount of Rs.1,64,280/- (Rupees one lakh sixty four thousand two hundred and eighty only) has been generated.

4. Human Resource Development

During this period, in the junior level training course, (Laboratory Animal Technicians Training Course), there were 7 participants and in the senior level supervisory training course, (Laboratory Animal Supervisors Training Course) there were 9 participants who underwent training in Laboratory Animal Sciences. In addition, an ad-hoc training was also given to 59 candidates for a period varying from one week to 4 weeks. These include 30 Ph.D scholars of institute who underwent Special training course.

The Centre celebrated World Laboratory Animal Day on 24th April 2012 and organized a National Symposium on “Laboratory Animal Sciences in the New Millennium-Challenges and Solutions” in collaboration with Laboratory Animal Science Association of India (LASAI), Federation for Indian Animal Protection Organizations (FIAPO), Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), ICMR and Indian Pharmaceutical Society (IPS). About 250 members from various parts of the country representing private and government organisations had participated. Dr. Dave Andersen, Director, Washington National Primate Research Centre (WNPRC), Seattle, USA was the special guest of honour and key-note speaker. During these celebrations, well known laboratory animal personnel were also felicitated.

Table 1. Details of Breeding and Supply of Different Species and Strains of Laboratory Animals of NCLAS April 2012 to March 2013

Sl. No	Species	Strain or Breed	Stock as on	Total number of animals							Balance as on
				Bred during the period	Available	Supplied to NIN	Supplied to other institutions	Supplied Total	Died	Disp.	
1	Mouse	BALB/c (in bred)	573	5138	5711	29	4480	4509	37	248	917
		C57BL/6J (in bred)	642	1972	2614	94	1171	1265	486	…	863
		NIH (S) Nude (in bred)	159	444	603	29	129	158	207	30	208
		NCr. Nude	179	581	760	3	284	287	208	…	265
		FVB/N (in bred)	151	102	253	…	25	25	30	50	148
		Swiss (in bred)	1553	4838	6391	120	4753	4873	660	…	858
2	Gerbils	…	4	82	86	…	…	…	…	…	86
3	G.Pig	Dunken Hartley (white)	358	1275	1633	…	1004	1004	138	119	372
		NIH Colour	216	428	644	…	412	412	55	105	72
4	Rabbit	New Zealand (white)	78	275	353	83	90	173	49	7	124
5	Monkey	Rhesus	18	…	18	…	…	…	…	…	18
Total			3931	15135	19066	358	12348	12706	1870	559	3931

Table 2. Details of breeding and supply of different species and strains of laboratory animals during for the month of April 2012 to March 2013

Sl. No	Species	Strain or Breed	Stock as on	Total number of animals							Balance as on
				Bred during the period	Available	Supplied to NIN	Supplied to other institutions	Supplied Total	Died	Disp.	
1	Rat	CFY/NIN (inbred)	122	74	196	…	…	…	65	45	86
		Fischer 344 N (inbred)	422	309	731	132	87	219	5	130	377
		Holtzman (inbred)	94	60	154	…	…	…	66	30	58
		SD (Sprague Dawley)-Outbred	897	2096	2993	496	1519	2015	...	300	678
		Wkyoto (inbred)	66	46	112	…	8	8	22	18	64
		WNIN (inbred)	3171	6542	9713	575	6415	6990	16	448	2259
2		WNIN / Gr-Ob	905	598	1503	90	…	90	118	467	828
3		WNIN / Ob-Ob (inbred)	1042	378	1420	109	…	109	127	402	782
4		SD NIN Nude	323	66	389	42	…	42	55	140	152
5	Hamster	Golden (inbred)	411	660	1071	82	438	520	80	65	406
6	Sheep	...	1	…	1	…	…	…	...	1	…
Total			7454	10829	18283	1526	8467	9993	554	2046	5690
Table-1 (Total)			3931	15135	19066	358	12348	12706	1870	559	3931
Table-2 (Total)			7454	10829	18283	1526	8467	9993	554	2046	5690
Grand Total			11385	25964	37349	1884	20815	22699	2424	2605	9621

Table 3. Stock Feed Supplied from NCLAS April 2012 to March 2013

Month	(A) Diet formulated protein %		(B) Govt. Institute Supply Protein %		(C) Private Institute Supply protein %		(D) Internal Supply Protein %	
	20 %	14 %	20 %	14 %	20 %	14 %	20 %	14 %
April-2012	3850	780	1332	670	221	--	2485	545
May 2012	4500	1140	1485	600	747	--	2925	395
June 2012	5350	1260	1954	850	325	3	2834	475
July 2012	5500	1260	1290	400	900	--	3150	460
August 2012	6250	1140	1820	700	480	9	3870	460
September 2012	6150	1080	2086	1100	880	--	3060	315
October 2012	6750	960	1715	200	965	0	3880	270
November 2012	6550	960	1542	700	660	105	3155	440
December 2012	4250	720	1485	250	1175	--	2435	510
January 2013	5150	900	1350	170	65	17	2905	550
February 2013	3600	600	1725	360	310	--	2672	295
March 2013	4700	480	955	100	905	--	2840	343
Total in Kgs	62600	11280	18739	6105	7633	134	36211	5058
	73880		24844		7767		41269	

On invitation, Centre also conducted three orientation programmes on animal experimentation for Ph.D research guides and students from Osmania University Hyderabad on 4th September, 2012, for research guides of NTR Health University, Vijayawada on 12th July 2012 and students of Apollo Hospital in Hyderabad on 7th November 2012.

The staff of the centre had participated at various national meetings and symposia on Laboratory Animals especially brain storming meetings in Indian National Science Academy (INSA) to discuss and finalize the recommendations on the New Animal Welfare Act (30th April, 2012) following a brain storming meeting on "Man, Animal and Science" in New Delhi (15th September, 2011).

B. RESEARCH ACTIVITIES

1 ESTABLISHMENT OF BASELINE VALUES OF BODY COMPOSITION AND BLOOD PRESSURE IN DIFFERENT SPECIES OF LABORATORY ANIMALS MAINTAINED AT NCLAS – A STUDY ON MICE STRAINS

The centre has been providing different species and strains of laboratory animals for in house use as well as to other organizations and also for preclinical toxicology testing. As a part of the activity of the centre, it was decided to establish the normal values of the animals maintained at the facility. Determination of base line values like body composition, locomotor activity, blood pressure and clinical chemistry parameters in rat strains maintained at the centre have completed. Last year, the work pertaining to the measurement of the base line values of Guinea pigs (G.pigs) and hamster in-terms of their body composition and clinical chemistry parameters got completed. The study was subsequently extended to New Zealand white rabbit strain maintained at the centre. During the year 2010-11 the study with an aim to establish the body composition [lean body mass, fat, fat%], by TOBEC, bone mineral density (BMD) and bone mineral concentration (BMC) by DXA] of rabbits of both sexes had initiated.

For the year 2011-12, we have analyzed four mice strains viz., Swiss albino, BALB/c and C57BL, maintained at our centre. Body composition parameters like LBM, fat, fat% and derived parameters like FFM, BMC and BMD levels were measured using DXA (Hologic QDR model, 1000 Series). The measurement of hematology and clinical chemistry parameters have been completed. Statistical analysis of the data is in progress. After completion of the statistical analysis of the parameters studied, a monogram on the animal species maintained at our centre will be prepared in terms of their body composition, blood pressure, hematology and clinical chemistry parameters. This monogram will be a ready reckoner to the research students/scientist who plan to carry out their research.

IX PRE-CLINICAL TOXICOLOGY RESEARCH CENTRE

1 PRE-CLINICAL EFFICACY (IBD, ANTI- INFLAMMATORY) AND SAFETY EVALUATION OF NOVEL PEPTIDE GENOPEP 4 (ISSAR 4)

Inflammatory bowel disease (IBD), a major health problem in the developed world comprises i) ulcerative colitis (UC) and (ii) Crohn's disease (CD) the chronic relapsing and remitting inflammatory disorders of the gastrointestinal tract. The incidence of UC is more than CD and it affects only the large bowel, whereas CD can affect any part of the gastrointestinal tract and may lead to colon cancer. The general symptoms of UC are malaise, lethargy, and anorexia accompanied by diarrhea with blood and mucus, abdominal discomfort, and fever. The underlying immune-pathogenesis of IBD is not clear. However UC and CD are characterized by activation of macrophages and T lymphocytes, pro-inflammatory cytokines. In the recent past, anti inflammatory properties of interleukin10 (IL-10) located in the lining of the intestines were extensively studied and their beneficial role through immune regulatory activity was reported .The reports on administration of 10N peptide[1] in Murine Model of Ulcerative Colitis: IL-10 Deficient Mice have shown protective effect against the colitis. The ISSAR Pharmaceuticals Pvt. Ltd. had developed IS 217 peptide which is similar to 10N peptide with an intention[2,3] to promote it as a therapeutic potential peptide in Ulcerative Colitis.

METHODOLOGY

The Efficacy potential has been determined in IBD –Dextran Sulfate Sodium (DSS) induced Ulcerative colitis (UC) rat model as it is a functional and established model at NIN.

Pilot study

The pilot study has been conducted to determine the dosage schedule of the ISSAR AP10-IBD peptide. In this study, the three months old male *Sprague dawley* rats (n=30) were selected and divided in five groups: (i) Non-colitic control (ii) UC control (iii) UC+ test compound 0.3 mg dose (iv) UC+test compound 0.6 mg dose (v) UC+test compound 0.9 mg dose. Except Non-colitic group all other group of animals were administered orally with 4% DSS in sterilized drinking water for 11 days to induce Ulcerative colitis (UC). In addition, the animals of group III, IV and V have been subjected for treatment with ISSAR AP10-IBD peptide by IV route two days before the induction of UC and continued daily for 14 days in various dose levels. Apart from monitoring daily body weight, food and water intake, DAI (Sum of Body weight, stool consistency and presence of faecal occult blood / 3) was also calculated. After 11 days of induction of colitis, all animals were fasted overnight and subject to gross necropsy to collect entire colon from colorectal junction to anal verge. The length of colon was measured and the colon was opened longitudinally and a portion of distal colon was immediately fixed in 10% neutral buffered formalin for further histopathological analysis.

Final study

In the final experiment a study was designed to assess the efficacy potential of ISSAR AP10-IBD peptide with the dosage schedule which can minimize the adverse events. In this study, the three months old male *Sprague dawley rats* (n=70) were selected and divided in seven groups: (i) Non- UC control (ii) UC control (iii) G-0.3mg/kg IV once in a day (iv) G-0.6mg/kg IV once in a day (v) G-0.9mg/kg IV once in a day (vi) G-0.6mg/kg SC thrice in a week and (vii) G-0.6mg/kg IV twice in a week. Except Non-colitic group, all other group of animals were administered orally with 4% DSS in

sterilized drinking water for 11 days to induce Ulcerative colitis (UC). In addition, the animals of group III, IV and V have been subjected for pre treatment with ISSAR AP10-IBD peptide by IV route two days before the induction of UC and continued daily for 14 days in various dose levels. Whereas animals of group VI were subjected for treatment with ISSAR AP10-IBD peptide by SC route thrice in a week and animals of group VII were subjected for treatment with ISSAR AP10-IBD peptide by IV route twice in a week, continued for 14 days. Body weight, stool consistency and presence of faecal occult blood or gross rectal bleeding were recorded daily for each animal. These parameters were used to calculate an average daily Disease Activity Index (DAI) according to the criteria proposed by cooper et al,.After 11 days of induction of colitis, all animals were fasted overnight and subject to gross necropsy to collect entire colon from colorectal junction to anal verge.

The length of colon was measured. From each group half of the animal samples were subjected to histopathological analysis and half of the animal samples for biomarker analysis. The colon was opened longitudinally and a portion of proximal and distal colon was immediately fixed in 10% neutral buffered formalin for further histopathological analysis and from remaining half of the animals, Mucosa was gently scraped with a microscopic slide and the collected mucosal samples were snap-frozen in liquid N2 and stored at -80°C for the biochemical determinations. Myeloperoxidase (MPO) activity, a marker of neutrophil infiltration into the colon, was measured as described as a one unit of MPO activity is corresponds to the activity required to degrade 1 μ mole H_2O_2 in minute at 25 ^{0}c. The content of TNF- α and IL-1β was determined by ELISA. The results were expressed as concentration per mg protein.

RESULTS

Pilot study

1. Rats given 4% DSS in drinking water for 11 days developed symptoms of acute colitis without mortality. In comparison to non-colitic controls, DSS-administered rats developed loose stools after the 2nd or 3rd day, which turned into diarrhoea on the 5th day. By the 10th or 11th day, gross rectal bleeding was observed. Based on the clinical symptoms (weight loss, stool consistency and intestinal bleeding), the DAI was assessed and the severity of colitis was calculated every day. The DAI became progressive from the 2nd day after DSS treatment and peaked between days 9 and 11 depending upon the treatment. Rats treated with 0.6 mg ISSAR AP10-IBD peptide had significantly less severity of the colitis as evidenced by a lower DAI score. The decrease in DAI score was apparent on days 9, 10 and 11 compared to other groups.
2. The severity of the colitis was also assessed by measuring the length of the colon which is an indirect marker of inflammation. DSS administration caused significant reduction in colon length in rats treated with 0.6 mg ISSAR AP10-IBD peptide compared with the non-colitic control rats.
3. There was no difference in food intake and body weight gain among the treated groups before the induction of colitis and after the DSS-induced colitis when compare with colitic group. Compared to the non-colitic control group, water consumption was higher in the colitic groups. However, there was no difference in the consumption of water in the colitic groups.
4. Based on Histopathology observations, damage to colonic mucosa is high in 0.9 mg dose when compared with 0.6 mg dose.

Final study

1. The indirect parameters like colon length, body weight change, food intake, water intake and DAI did not alter in any group when compared with UC control group, irrespective of the drug treatment.
2. The Histopathological observations suggests that 0.6 mg/kg dose by SC weekly thrice dose was effective in IBD.

3. Inflammatory status of the colon was assessed biochemically by determining MPO activity, an enzymatic marker of neutrophil infiltration. DSS treatment significantly increased colonic MPO activity compared to non-colitic control group. However, the treatment with 0.6 mg/kg dose by SC weekly thrice significantly decreased the MPO activity, suggesting that ISSAR AP10-IBD peptide exerts an anti-inflammatory effect by reducing the neutrophil infiltration into the colonic mucosa.
4. It is known that TNF-α and IL-1β are the major inflammatory mediators in the pathogenesis of UC and they are produced from both inflammatory cells and mucosal epithelial cells during inflammation. 11 days after colitis induction by DSS administration, the tissue levels of TNF-α and IL-1β were observed to be significantly higher than that of the non-colitic control rats. 0.6 mg/kg dose by SC weekly thrice significantly suppressed DSS-induced increase in TNF-α and IL-1β levels.

CONCLUSION

About 0.6 mg dose was found to be effective and hence efficacy study was done based on the results of the pilot study. The administration of 0.6 mg/kg of test compound -IS 217 by thrice weekly for two weeks by SC route had demonstrated anti-inflammatory property in Inflammatory Bowel Disease, which was evaluated by DAI, Histopathology and Biochemical markers. Whereas the Intravenous route at the above dosage schedule had also reduced inflammatory properties but it couldn't significantly correlate with biomarkers. In higher dosage, the anti inflammatory properties were recorded with mortality.

2 PRE-CLINICAL TOXICOLOGY STUDY OF RECOMBINANT INTERFERON BETA-1B (IFN ß1B)

Interferon is a naturally occurring protein with a molecular weight ranging from 15 to 21 Kilo Daltons. Among the thre major classes of interferon viz. alpha, beta and gamma, M/S Sudershan Biotech Limited (SBL) had developed Interferon beta – 1b with an intention to promote in the treatment of multiple sclerosis using recombinant DNA technology. The Pre clinical toxicity investigation (Acute and sub–chronic) had been conducted as per DBT/Schedule Y of DCGI guidelines.

METHODOLOGY

1. The acute toxicity tests has been undertaken in Mice & Rats with single exposure of test compound by i. Intramuscular, ii. Subcutaneous route in 10 times of intended recommended therapeutic concentration. This is followed by observation by activity and lethality in addition to bi–weekly monitoring of live phase, cage side and physical observations till 15th day of post exposure. All the animals were euthanized on i.e., after test compound exposure.
2. Sub chronic Toxicity investigation in New Zealand White rabbits (35 animals and Swiss albino mice (60 animals) had been conducted by exposing in three different doses viz., i. Therapeutic dose (TD), ii. Average dose (five times of TD), High Dose (ten times of TD) by Subcutaneous route. This is followed by monitoring live phase, cage side observations, physical and neurological activities bi–weekly. The clinical chemistry and hematology profile had been assessed on 48 hours and 15th day of post exposure to test compound. The gross necropsy and histopathological investigation of all organs had been undertaken. The allergenicity potential of the product was evaluated by physical examination for symptoms and signs related to allergenicity and by IgE quantification by ELISA.

RESULTS

1. No mortality was observed to test material administered by acute exposure to Intramuscular or Subcutaneous route in 10 times of intended therapeutic dose in mice rats.
2. In sub chronic toxicity study, mortality was recorded in two female mice received high dose of test compound. However, in rabbits there was no mortality. There were no differences in body weight gains, live phase, physical activity and neurological activity test groups as compared to control and throughout the study period. The clinical chemistry parameters viz., blood glucose levels, kidney and liver function tests were found to be in normal range in all groups of animals exposed to the test compound when compared to vehicle control. Among hematological parameters except for hemoglobin all other parameters were within normal range. Gross necropsy and histopathological evaluations were essentially unremarkable and do not appear to be related to test compound usage at doses and time points administered.

   A Dose dependent increase in serum total IgE levels was observed after 14 doses of Interferon beta – 1b (IFN β-1b).However, fifteen days after withdrawal of treatment, total IgE decreased in AD group (7 folds) and HD group (two and half folds), while in therapeutic dose the IgE was below detectable level and similar to vehicle control group.

CONCLUSION

The No Observable Adverse Effect Level (NOAEL) of test compound was observed after administration of 14 doses 10 times more than equivalent human dose (3 mg / adult) on alternate day for 28 days in mice and Rabbits.

3 PRE CLINICAL TOXICITY EVALUATION OF TRANSGENIC COTTON (CRY1AC EVENT-1& CRY1 EC EVENT-24)

Genetic modification using recombinant DNA technology is leading to the development of new agriculture plants with better quality and quantity. M/S *J. K Agri Genetics Ltd.* had developed Bt cotton containing a stack of *Cry1Ac (event –1) & Cry1EC (event–24) proteins* for insect resistant trait. This will help farmer in reducing the pesticides sprays and will prevent loss of production. Despite the benefits and economic advantages of such crops, biosafety issues of such products need to be evaluated. Therefore the following investigations has been undertaken to assess i. Assessment of Allergenicity of Bt. Proteins, ii. Acute Oral Toxicity of Cry 1 Ac, Cry 1 EC and Combination Cry1Ac and Cry1EC, iii. Sub – Chronic Toxicity Study of Bt cotton lyophilized Leaf and Seed.

METHODOLOGY

1. Allergenicity of Bt. Cotton containing Cry1Ac Event 1 and Cry1EC Event 24 recombinant proteins in Bt.Cotton had been assessed by (A) In vitro Pepsin Digestibility Assay in Simulated Gastric Fluid (SGF), (B) Protein Thermal stability using the SDS–PAGE gel analysis and (C) Bioinformatic analysis.
2. Acute toxicity test (14 days) in *Swiss albino* mice and *Sprague Dawely* Rats was carried out by exposing test Proteins [Cry1Ac (Bt.Ac), Cry1EC (Bt.EC)] in a concentration of 2000 mg/kg. the control group of animals received Phosphate buffer saline and vehicle control received lysate. The maximum value to achieve the maximum concentration was maintained at 0.25ml. This was followed by observation by activity and lethality in addition to bi–weekly monitoring of live

phase, cage side and physical observations till 15th day of post exposure. All the animals were euthanized on i.e., after test compound exposure.

3. The sub-chronic toxicity study was conducted by feeding lyophilized samples of Bt. Cotton (22.5g/kg) Leaf and Seeds in 120 *Sprague dawley* rats, aged 4–6 weeks, weighing 150 – 180g. The animals were divided randomly into six groups (Control, Non Transgenic Seed (NTS), Transgenic Seed (TS), Control, Non Transgenic Leaf (NTL) and Transgenic Leaf (TL)) to receive the Seed (TS) and another three groups to receive the Leaf daily for 90days. This was followed by monitoring the animals bi–weekly for live phase, cage side, physical and neurological parameters. The clinical chemistry profile, haematology, serum IgE levels, gross necropsy and histopathological observations of all organs were carried out after 48 hrs and 15th day of post exposure to normal, (NTS) and (TS) diet.

RESULTS

1. The results of the pepsin digestibility assay showed that both the recombinant proteins were rapidly degraded by pepsin in SGF and 90% digestibility was achieved at < 0.5mins.
2. Insect bioassay of heat treated Cry1Ac and EC recombinant proteins showed that at a temperature of 95°C the mortality of target insects of both the proteins was zero.
3. None of the results from the three bioinformatics searches with the Cry1AcJK sequence met the criteria that might suggest potential allergenic cross–reactivity.
4. There were pre–terminal deaths in mice received lysate [(10%) on 3rd day of post exposure], Cry1Ac [(10%) on 11th day of post exposure] and Cry1 EC [(10%) on 2nd day of post exposure]. In surviving animals, there were no differences in body weight gain, live phase and physical activity throughout the study period. In rats, there was no mortality.
5. There was no mortality in any group of animals fed with normal diet, Bt Cotton Seed and Leaf till end of the experimental period. There was no significant reduction in body weight gain in test groups non transgenic and transgenic. No changes in live phase, physical activity and neurological activity between the control and test groups were recorded throughout the study period. Clinical chemistry and hematology profile in blood / serum samples collected after 48hrs and 15th day of post exposure, were in norma;l range though there were significant changes observed. The total serum IgE levels of all the tested groups were comparable to control group at midterm as well as final term euthanizations. At necropsy, no gross lesions were found in any organ in all groups. No histopathology changes were recorded in organs of animals between control and test groups. Serum IgE and IgG levels were also monitored.

CONCLUSIONS

- Cry1Ac Event 1 and Cry1EC Event 24 recombinant proteins were rapidly digested by pepsin.
- The Bioassay and SDS–PAGE analysis the Cry1Ac Event 1 and Cry1EC Event 24 recombinant proteins was heat labile.
- The results from all the three search methods indicate no significant matches of the test proein sequences with any allergenic proteins.
- There were no differences in body weight gain, live phase and physical activity throughout the study period. The preterminal death in Mice are not test compound related.
- No mortality was observed after administration of test material continuously for 90 days. There was no abnormality in clinical chemistry and hematology profile in all three groups. The histopathological findings were not related to test compound exposure. The serum IgE levels were comparable to control group.

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 dessimination 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 76 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 1679 journals received collectively at 30 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 29,829 e-journals with links to full text at publisher sites and provides free access to full-text of 3618 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 1812 Journals

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

e) The following equipments were procured for the library.

i) HP PC	-	7
ii) UPS	-	2
iii) Vaccum Cleaner	-	1

New Journals Added

Foreign Journals

1 Annals of Clinical Biochemistry
2 B B A – General Subjects
3 Ecology of Food and Nutrition
4 Experimental Biology and Medicine
5 Genes and Nutrition
6 Gerontology
7 Human and Experimental Toxicology
8 International Journal of Food Safety, Nutrition and Public Health
9 Investigative Ophthalmology and Visual Science
10 Journal of Early Childhood Research
11 Journal of Health Service Research and Policy

12 Journal of Medical Screening
13 Nutrition and Dietetics
14 Obstetric Medicine
15 Phytomedicine
16 Protein and Peptide Letters
17 Proteomics
18 STM – Current Trends in Clinical Research
19 STM – Journal of Nanoscience, Nanoengineering and Applications
20 STM – Research and Reviews A Journal of Biotechnology
21 STM – Research and Reviews: Journal of Genomics and Proteomics
22 STM – Research and Reviews: A Journal of Herbal Science
23 STM – Research and Reviews: A Journal of Life Sciences
24 STM – Research and Reviews: Journal of Medical Science and Technology
25 STM - Research and Reviews: Journal of Veterinary Science and Technology
26 Tropical Doctor

The following library services were expanded as detailed below:

1. New additions

Books	. . . .	276
E-Books	.....	36
Reports	. . . .	210
Journals (New Subs.)	. . . .	26
Thesis / Dissertations	. . . .	7
CDROMS	. . . .	26
PC Quest CD's 12		
General CD's . . . 14		

2. Other activities

Journals Bound	. . . .	1,920
Visitors using the Library	. . . .	1,056
Circulation of Books/Journals etc	. . . .	1, 410
No. of E-mails sent outside	. . . .	1, 750
No. of E-mails received	. . . .	19,210
Photocopying (No. of pages)	. . . . .	2,28,374
Number of Annual Reports mailed	. . . .	468
No. of INTERNET Searches provided	. . . .	100
No. of Reprints sent	. . . .	60
Proquest Full Text Database searches provided. . . .		76

3. Total library collections

Books	. . . .	18,082
Journals (Bound Volumes)	. . . .	37,543
Journals subscribed for 2012	. . . .	383
Journals received (Gratis/Exchange)	. . . .	320
Microforms (Microfiche)	. . . .	1,080
Slides	. . . .	280
Reports	. . . .	13,371
Theses & Dissertations	. . . .	394
MEDLINE CDROMS Discs	. . . .	383
Current Contents on Diskettes with abstracts. . . .		664
Proquest (Full Text E-Journals) on CD ROMS. . . .		495
General CD's		236

Ph.D. PROGRAMMES

Ph.D. AWARDEES

S. No	Research Scholar	Title of the Thesis	Award Year	University
1.	K.Anand Kumar	Maternal Vitamin B12 and / or Folate Restriction Induced Changes in Body Adiposity, Hyperglycemia and Insulin Resistance in Wistar Rat Offspring : Molecular Basis of the Changes	2012	Osmania
2.	A.Satyanarayana	Biochemical, Molecular and Nutritional Aspects of Diabetic Retinopathy	2012	Osmania
3.	Manisha Ganeshan	Developmental origins of adiposity and Insulin resistance : Role of peri / postnatal Manganese status and high fat feeding in later life	2012	Osmania
4.	P. B. Sainath	Insulin signaling mechanisms in the hypothalamus (brain) of the NIN obese mutant rat: WNIN / Ob	2012	Osmania
5.	P. Yadagiri Reddy	Biochemical studies on obesity induced cataractogenesis using WNIN-obese rat model	2013	Osmania

RESEARCH SCHOLARS REGISTERED FOR Ph.D.

S.No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
1	Jitendra K. Sinha (2009)	Reduced longevity of obese rats : Role of altered support, neurochemical and oxidative damage in brain.	Dr. M. Raghunath	Osmania
2	Anju E. Thomas (2010)	Fetal programming for neuro-muscular skeletal development in the rat offspring – Role of antenatal and perinatal Mg deficiency	Dr. M. Raghunath	Osmania
3	K. Nagabhushan Reddy (2011)	Dietary phytate–Zinc ons: "Role in suppressing colon cancer"	Dr. M. Raghunath	Osmania

S.No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
5	Mehraj (2009)	Role of UPP in vit –D deficiency induced muscle atrophy and hypo-insulinemia.	Dr. Ayesha Ismail	Osmania
6	Bindu (2010)	Polyphenol rich dietary ingredients as novel sources of proteasome inhibitors and their role as anti cancer agents.	Dr. Ayesha Ismail	Osmania
7	Little Flower Augustine (2007)	Stress, allostatic and micronutrient status among higher secondary students: Impact of dietary advice	Dr. K. Madhavan Nair	Osmania
8	Swarnim Gupta (2008)	Dietary diversification of Indian diets to improve iron bioavailability	Dr. K. Madhavan Nair	Osmania
9	N. Pallavi (2010)	Regulatory role of zinc in hepcidin mediated iron metabolism	Dr. K. Madhavan Nair	Osmania
10	Y.Sravanthy (2010)	Effects of prenatal iron supplementation on iron-zinc homeostasis and placental zinc transporters : Studies in pregnant women and in BeWo cell lines	Dr. K. Madhavan Nair	Osmania
11	A. Kiran Kumar (2012)	Metabolic response of zinc depletion and excess in contrasting cells: Studies in osteoblasts, myocytes and enterocytes.	Dr. K. Madhavan Nair	Osmania
12	P. Ravindranath (2011)	Purification, characterization and primary structure elucidation of human milk factor that enhances iron absorption	Dr. P. Raghu	Osmania
13	Purna Chandra (2012)	Manipulation of dietary fat to enhance carotenoid bioavailability and bioconversion to vitamin A: Development of mechanism based strategies.	Dr. P. Raghu	Osmania
14	A.Vijayendra chary (2011)	Maternal vitamin D status. Vit D receptor expression and their link with CD23/CD21interaction and regulatory T cell function.	Dr. R. Hemalatha	Osmania
15	N. Himaja (2012)	Effects of Fos coated probiotics on fetal immune-programming and other health benefits.	Dr. R. Hemalatha	Dr. NTRUHS
16	N. Padmaja (2012)	Link between diet, inflammation and pregnancy outcome and effect of oral probiotics.	Dr. R. Hemalatha	Dr. NTRUHS
17	Daniella Chyne (2012)	Studies on the biodiversity of food resources in Meghalaya.	Dr. R. Ananthan	Osmania
18	M.A. Patil (2010)	Studies on Diabetic Complications: Evaluation of Animal models and Role of Dietary agents	Dr. P.Suryanarayana	Osmania
19	Naga muralidhar (2011)	Genetic and epigenetic approach towards obesogenicity in a rat	Dr. K. Rajender Rao	Osmania

S. No	**Research Scholar** (Year of joining)	**Title of the Thesis**	**Supervisor/ Guide**	**University**
20	Priyanka Shankar (2006)	Effect of high fluoride and low calcium on bone metabolism in rats	Dr. Arjun L. Khandare	Osmania
21	N. Aparna (2011)	ß-ODAP induced neurotoxicity and changes in glutamate receptor gene expression in rat	Dr. Arjun L. Khandare	Osmania
22	M. Ankulu (2011)	Effect of excess nitric oxide in the patho physiology of motor neuron degeneration in neuroalthyrism.	Dr. Arjun L. Khandare	Osmania
23	S.Alekhya (2012)	Identifying microbiological and hygienic factors affecting safety of stress foods and addressing them through vendor education.	Dr. V.Sudershan Rao	Osmania
24	Madira Soudarya Lakshmi (2008)	Establishment of propayable cell lines from Adipose tissue of adult WNIN Obese Mutant Rat (WNIN/Ob and WNIN/GR-OAB)	Dr. Vijayalakshmi Venkatesan	Osmania
25	Himadri Singh (2010)	Establishment of propayable cell lines from Pancreas (Ducal Epithelial Cells) of Adult WNIN Obese Mutant Rats (WNIN/Ob and WNIN/GR-Ob)	Dr. Vijayalakshmi Venkatesan	Osmania
26	Sowmya Sharma (2011)	Modeling the developmental origins of health diseases in the mouse embryonic stem cells (mESCs) – Cellular, molecular/epigenetic approaches	Dr. Vijayalakshmi Venkatesan	Osmania
27	Deethu Sara Varghese (2008)	Assessment of body composition in Indian females using different techniques.	Dr.Y. Venkata Ramana	Osmania
28	Anupama Tyagi (2009)	Anti inflammable potential & n-3 PVFA in experimental Biochemical and molecular mechanism.	S. Ahmed Ibrahim	Osmania
29	Anil Sakumari (2010)	Modulation of adipose tissue inflammation and function of dietary n-3 pvfa: Potential role in metabolic syndrome.	S. Ahmed Ibrahim	Osmania

S. No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
30	J. Sugeetha (2012)	Impact and dietary n-3 and n-6 pufa on the progression of non-alcoholic fatty	S. Ahmed Ibrahim	Osmania
31	B.Shankar Anand (2009)	Understanding the role of T cells and secreted cytokines in the Development of insulin resistance during obesity	Dr.Sudip Ghosh	Osmania
32	Golla Venkateswarlu (2010)	Role of dietary fatty acids in inducing endoplasmic reticulum stress in stromal vascular cells : Implications in the development of obesity associated insulin resistance	Dr.Sudip Ghosh	Osmania
33	K.Sandeep Kumar (2011)	Role of miRNA in the development of obesity and diabetes	Dr.Sudip Ghosh	–
34	Srivani Muthuswamy (2005)	Understanding the role of pro-inflammatory cytokines in the development of insulin resistance and diabetes using WNIN-ob obese rat models (under flashing project)	Dr. Nasreen	Uuniv. of Hyderabad
35	J. Vahini (2011)	Studies on assessment, identification and modification of glycemic index in dietes commonly consumed by people.	Dr.K. Bhaskara chary	Osmania
36	N. Naveena (2011)	Studies on polyphenols in some plant foods as a source of antioxidants	Dr.K. Bhaskara chary	Osmania
37	Agatha Betsy (2008)	Assessment of dietary intake of chemicals contaminants among various socioeconomic sections of Hyderabad.	Dr. Kalpagam Polasa	Osmania
38	N. Chetan (2009)	Evaluation of herbal neutraceutics: Anti-atherosclerotic activity.	Dr. Dinesh Kumar	Osmania
39	M. Chalamaiah (2010)	Fish egg hydrosylation	Dr. Dinesh Kumar	JNTU (H)
40	V.Varsha (2011)	Evaluation if the impact of genetic polymorphism on the pharamacodyanamic activity of commonly prescribed anti hypertensive drugs	Dr. Dinesh Kumar	JNTU(H)
41	V.Sudhakar Reddy (2009)	Role of small heat shock proteins in diabetic complications: Modulation by nutritional and dietary factors.	Dr. Bhanuprakash Reddy	Osmania
42	Sarin Sara Jose (2010)	Studies on nutritional assessment of diabetic complications.	Dr. Bhanuprakash Reddy	Osmania
43	K.Ashok Reddy (2010)	Studies on aldose reductase in human cancer and chemo-drug resistance: Role of dietary AR inhibitors as adjuvants in chemotherapy.	Dr. Bhanuprakash Reddy	Osmania

S. No	Research Scholar (Year of joining)	Title of the Thesis	Supervisor/ Guide	University
44	S. Vishwaraj (2012)	Proteomics of diabetic complications	Dr. Bhanuprakash Reddy	Osmania
45	Sneha Jakhotia (2012)	Role of RBP4 in the pathophysiology of diabetes	Dr. Bhanuprakash Reddy	Osmania
46	K. Shruthi (2012)	Role of CTRPs in diabetes and retinal degeneration	Dr. Bhanuprakash Reddy	Osmania
47	T. Shalini (2012)	Assessment of health and nutritional status of urban geriatric population	Dr. Bhanuprakash Reddy	Osmania
48	Anuradha Challa (2011)	Impact of dietary facts rich in n-6 and n-3 poly unsatured fatty acids on adiposity and insulin resistance in diet induction obese rat model: a missing molecular link with vit a metabolism	Dr. A. Vajreswari	Osmania
49	Anantha Krishna Vemuri (2011)	Impact of nutritionally superior high oleic acid varieties of mustard oil on lipid metabolism	Dr. A. Vajreswari	Osmania
50	M.Srujana (2012)	Effect of pesticide exposure among the farm children and their mothers on the various biochemical parameters associated with reproduction, neurotoxic enzymes, oxidative stress and impact on the micronutrient status	Dr.J.Padmaja Rambabu	Osmania

INSTITUTIONAL STAFF REGISTERED FOR Ph.D.

S. No	Name of the Staff	Title of the Thesis	Supervisor	University
1.	Dr.A.Laxmaiah (2008)	Assessment of prevalence of overweight/obesity, hypertension and type 2 diabetes among 20-60 year Urban population in Hyderabad	Dr.B.Sesikeran	Dr.NTR University of Health Sciences
2.	Ajumera Rajanna (2011)	Embryonic stem cells as model system ro study the developmental origin of health in micronutrients-obesity/type 2 diabetes	Dr. Vijayalakshmi Venkatesan	Osmania
3.	K.Mangthya Naik (2012)	Studies gastro- protective effects of Naga King chili	Dr. R. Ananthan	Osmania

AWARDS/ HONOURS CONFERRED ON SCIENTISTS

Name of the Scientist	Awards/ Honour Conferred
Dr.MP.Rajendra Prasad	"Certificate of Appreciation" for outstanding contribution on the project 'Creation of Demand for Millet Foods through PCS value-chain' from National Agricultural Innovation Project (NAIP), ICAR at the XXI Meeting of the ICAR Regional Committee No.II held at NAARM, Hyderabad in the presence of his Excellency the Governor of Andhra Pradesh
Dr. M. Raghunath	Selected as a member, Board of Studies in Biochemistry, Sri Krishna Devaraya University, Anantapur for the period 2012 – 2015
Dr.G.M.Subba Rao	ICMR International Fellowship for Young Biomedical Researcher 2012 to pursue research and training at Department of Health, Behaviour and Society at Johns Hopkins Bloomberg School of Public Health, Baltimore, USA (Jan – Apr, 2013)
Dr. G. Bhanuprakash Reddy	Elected as a Fellow of AP Akademi of Sciences for the year 2012.

AWARDS/ HONOURS CONFERRED ON RESEARCH FELLOWS/ STUDENTS

Name of the student	Award/ Honour received
Ms.Little Flower Augustine	Ramanathan Prize Award for best oral presentation in the Free Communications for paper titled "Low grade inflammation in stressful life events is not associated with hypo-ferremia among adolescent boys" at the 44th National Conference of Nutrition Society of India, Tirupathi Chapter, Tirupathi
Ms.Priyanka Shankar	Ramanathan Prize Award for best oral presentation in the Free Communications for paper titled "Amelioration of adverse effects of fluoride toxicity on calcium homeostatis in low calcium treated rats" and Young Scientists' Junior Award in Experimental Nutrition for paper titled "Downregulation of osteoporlin and reduced biochemical properties in low calcium and fluoride treated rats" at the 44th National Conference of Nutrition Society of India, Tirupathi Chapter, Tirupathi
M. Chalamaiah	Best poster Prize for the presentation "Nutritional quality, chemical composition & antioxidant activity of eggs of major Indian fresh water fish species" in Food Technology category at the ICMR and DST sponsored International conference on "Food Technology for Health Promotion", held at JNU, New Delhi during 27-28th Dec. 2012

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

Name of the Scientist	Conference/ Workshop/ Seminar
2012	
Dr. G. Bhanuprakash Reddy and Ms. C. Akileshwari (SRF)	Mid-term Meeting of Indo-EU Funcfood Project, held at Stockholm, Sweden (May 23-25).
Dr.SSYH Qadri	The FASEB International Summit on “The Threat of Extremism to Medical Research: An International Summit”, held at National Jewish Health, Colarado, USA. Made a presentation on “Animal Rights Extremism in India” in the Session on “International Historical Perspectives” (June 11-12).
Dr.Ayesha Ismail	Presented a paper on "Vitamin D deficiency induced muscle protein degradation occurs through the ubiquitin proteasome pathway" at the 15th Vitamin D workshop, held at Houston, USA (June 20 – 22).
Dr.B.Sesikeran	One-day Workshop on “Genetically Modified Animals for Food and Pharmaceuticals: Are we Ready?” held at Brussels, Belgium (July 3).
Dr.K.Madhavan Nair	Collaborators meeting as part of the Indo-US Collaborative project on ‘Bioavailability of iron and zinc in representative Indian and US diets/ Enhancing dietary iron and zinc bioavailability in Indian children’, at Baylor College of Medicine, Houston, Texas (Aug. 20-31). Collaborators meeting as part of the project on “Innovative strategies to promote early child development among low-income rural infants and preschoolers in India through multiple micronutrient fortification and early learning opportunities”, at University of Maryland Medical Center, Baltimore (Aug. 27-28).
Dr.R.Hemalatha and Dr. Manoj Kumar (ICMR-PDF)	Presented a Paper titled, "Synergistic effect of Non-steroidal Anti-Inflammatory Drug, Lactobacillus rhamnosus GG and Allium Sativum on PI3-kinase and Wnt Signaling on DMH induced Colon Cancer'', at the 11th International Conference on ‘Functional Foods and Chronic Inflammation: Science and Practical Application’ held at the San Diego University, USA (Aug. 23).
Dr.P.Suresh	Represented India as a National member and attended the International Council for Laboratory Animal Science (ICLAS) General Assembly meeting held in Bangkok, Thailand (Oct. 9). Asia and India Regional Committee meeting and International Consortium of the International Council for Laboratory Animal Science (ICLAS) organized in conjunction with the 5th meeting of the Asian Federation of Laboratory Animal Science (AFLAS) held in Bangkok, Thailand (Oct. 12).

Name of the Scientist	Conference/ Workshop/ Seminar
2013	
Dr.K.Madhavan Nair	Talk on " Fortification and bioavailability of micro nutrients: Possible options to combat hidden hunger" at the Plenary Session of Annual Scientific Session of Nutrition Society of Sri Lanka (Feb. 2-3).
Dr.Y.Venkataramana	Appointed as Temporary Adviser to the Regional Director by the World Health Organization Regional Office for South-East Asia (WHO-SEARO) to provide technical assistance as Sports Nutrition Expert to the Department of Health and Department of Sports, Yangon, Myanmar to provide nutrition advise and assist the National Sports Council, Myanmar who are preparing their athletes for the forthcoming South-East Asian Games-2013, Myanmar (Feb. 18-23).

WORKSHOPS/ CONFERENCES/ SEMINARS/ TRAINING PROGRAMMES HELD AT NIN

1. Brainstorming Session on Food and Inflammation organized by NIN in collaboration with Department of Biotechnology, New Delhi (April 10-11).
2. Training of Technicians for "National Programme for Prevention and Control of Fluorosis", organized in association with Directorate General of Health Services, Ministry of Health, Govt. of India, New Delhi (April 16-20).
3. In connection with World Laboratory Animal Day, A National Symposium on "Laboratory Animal Science in the New Millennium – Challenges and Solutions" was organized by National Centre for Laboratory Animal Sciences, NIN, ICMR in association with Committee for the Purpose of Control and Supervision of Experimentation on Animals, Laboratory Animal Science Association of India, DBT, DST, Indian Pharmacological Society and Federation of Indian Animal Protection Organisations (April 24-25).
4. Translational Workshop on "PCR-based Food Pathogen Detection Kits" developed under NIN-Bioserve joint initiative (April 26).
5. National Dissemination Workshop on Findings of NNMB Tribal Survey and Brain Storming Session on "Intervention Strategies for Prevention and Control of Double Burden of Disease" (May 23-24).
6. Training Programme on "Nutritional Assessment Techniques" for the project staff of M.S.Swaminathan Research Foundation, Chennai (June 11-22).
7. Training of Trainers for "National Programme for Prevention and Control of Fluorosis", was organized at NIN in association with Directorate General of Health Services, Ministry of Health, Govt. of India, New Delhi (July 19-20).
8. "Biodiversity Management for Alleviating Poverty and Malnutrition", organized in connection with XI Conference of the Parties to the Convention of Biological Diversity, by M.S. Swaminathan Research Foundation and National Institute of Nutrition (Oct. 10).
9. One-day Workshop on "Agricultural Cooperatives: Key to Feeding the World" (Theme of World Food Day – 2012) was organized in association with Association of Food Scientists and Technologists (Hyderabad Chapter) and Oil Technologists Association of India (Oct. 16).
10. International Conference on Recent Trends in *Lathyrus sativus* Research (Nov. 9-10).
11. Golden Jubilee Conference of Indian Dietetic Association. As part of the Conference, four pre conference workshop were organized on "Nutritional Epidemiology and diet survey", "Entrepreneurship in nutrition and dietetics", "Research proposal, design and biostatistics" and "Train the Trainer (workshop organized by RD Board, IDA) (Nov. 29).
12. 45th Annual National Conference of IDA-2012 on "Indian Diets and Health: Retrospect and Prospect", jointly organized by Indian Dietetic Association (AP Chapter) and National Institute of Nutrition (Nov. 30-Dec.1).
13. Training of the Trainers of National Programme for Prevention and Control of Fluorosis (Dec. 13-14).
14. Fiftieth Post-Graduate Certificate Course in Nutrition (Jan.3-March 15, 2013).

SERVICES RENDERED TOWARDS INCOME GENERATION

1. PATHOLOGY SERVICES

During the year, a total income of Rs. 6,16,555/- was generated from various projects of Institute's preclinical toxicology and surgical pathology and cytology samples analysation.

2. TRAINING PROGRAMMES

I. An amount of Rs. 6,60,000/- was generated from the tuition fee collected from the first and second year participants of 2 year MSc (Applied Nutrition) course (1st year – 17 and 2nd year-16 candidates).

ii. An amount of Rs. 1,42,000/- was generated from fifteen private candidates admitted to the regular training programme viz., Post Graduate Certificate Course in Nutrition (11) and Annual Certificate Cource on Endocrinological Techniques and their Applications (4).

INSTRUMENTATION SERVICES

List of Instruments installed by the instrumentation department during the year 2012-13

S.No	Name of the Instrument	Make	Model	Location
1	Digital Rotary Flash Evaporator	Genevac	EZ2 Envi	Ocular Biochemistry
2	Centrifugal Cell Disruptor	Omni, USA	BR 31467	Ocular Biochemistry
3	Micro Array Hybridization Oven	Sheldon	G2545A	Molecular Biology
4	Multicapillary Electrophoresis	Qiagen	QIAxcel advance Prio PLUS	Molecular Biology
5	Table Top Refrigerated micro Centrifuge	Hermle, Germany	Z216MK	Ocular Biochemistry
6	Water Purification System	Elga, UK	Pulse Purelab + Classic UV	Molecular Biology
7	UV-VIS Spectrophotometer	Dynamica, Australia	HALO-SB10	MSc Lab
8	Densitometer (Molecular Imager)	Bio-Rad, Hong kong	GS-800	Ocular Biochemistry
9	Double Beam Scanning UV-VIS Spectrophotometer	Shimadzu, Singapore	UV-2600	Ocular Biochemistry
10	Laser Dissection Microscope-upgradation	Leica, Germany	DFC450 camera, 4x & 100x Obj.	NIN/Common facility
11	PCR workstation	UVP	UV3	Molecular Biology
12	Biosafety Cabinet	Dyna, Pune	Class-II	
13	Microwave Digestion System	CEM	MARS 5	FDTRC
14	Ultra Sonic Bath	Medica	Equitron	FDTRC
15	Heating Block	Grant	QBH2	FDTRC
16	Electronic Balance-2nos.	Eagle	Silver 2	FDTRC
17	Tissue Homogenizer	Omni	THW 220V	FDTRC
18	Gas Chromatograph with Head Space Auto sampler	Perkin Elmer	Clarus 680 HS 40	FDTRC
19	Atomic Absorption Spectrometer	Shimadzu	AA7000	NIN/Common facility

S.No	Name of the Instrument	Make	Model	Location
20	Auto Gamma Counter	Perking Elmer	Wizard 2480	NIN/Common facility
21	Western Blot	Bio-Rad	SDS PAGE	Microbiology
22	Digital Balance	Sartorius	BSA 423SCW	Microbiology
23	Binary gradient HPLC systems with ELSD & FLD	Dionex	Ultimate 3000	Food Chemistry
24	Water Purification System	Millipore	Elix-Milli-Q	Food Chemistry
25	BOD incubators-2nos	Labtech	LIB-250M	Food Chemistry
26	Autoclave-2nos	Labtech	LAC-5100SD	Food Chemistry
27	Grossing Station	Yarco	YSI-222	Pathology
28	Binocular Stereo Microscope	Olympus	SZ-51	Pathology
29	Spectrum imaging system	Caliper	IVIS Spectrum	NCLAS
30	Electrophoresis	Bio-Rad	Power Pack 100	Lipid Chemistry
31	HPLC	Dionex	LPG 3400SD	Lipid Chemistry
32	Tissue Slicer	Harvard	OTS-4500	Stem Cell
33	RO Water Purification -2nos	Simens	1N37199617	NCLAS
34	GC-HS	Perkin Elmer	CLARUS680	Lipid Chemistry
35	Rotary Evaporator	Heidolph	HEI-VAP Pre	Endocrinology
36	PCR	Eppendorf	Pro384	Stem Cell
37	PCR	Eppendorf	Pro384	NCLAS

SCIENTIFIC PUBLICATIONS

A. PAPERS PUBLISHED IN SCIENTIFIC JOURNALS

1 Anupama Tyagi, Uday Kumar, Suryam Reddy,Santosh VS, Saazida B.Mohammed, Nasreen Z.Ehtesham, Ahamed Ibrahim: Attenuation of colonic inflammation by partial replacement of dietary linoleic acid with α-linolenic acid in a rat model of inflammatory bowel disease. Br. J Nutr.108:1612-1622,2012.

2 Arlappa N, Balakrishna N, Laxmaiah A, Brahmam GNV: Prevalence of anaemia among rural pre-school children of Maharastra, India. Indian J Community Health 24:4-8, 2012.

3 Ayesha Ismail, Bindu N, Shulagna Sharma, Chandana M, Mehrajuddin Bhat, Raghunath M:Proteasome Inhibitory potential of commonly consumed dietary ingredients. Int J Food Nutr Sci 1: 27-31, 2012.

4 Babu Geddam JJ, Radhakrishna KV, Ramalaxmi BA, Ravinder P, Balakrishna N: Expression of leptin in early placental development – its association with maternal nutritional status. Int. J Med Public health. 2:21-24, 2012.

5 Babu MS, Prabha TS, Kaul S, Al-Hazzani A, Shafi G, Roy S, Balakrishna N, Jyothy A, Munshi A. Association of genetic variants of fibrinolytic system with stroke and stroke subtypes. Gene. 495:76-80, 2012.

6 Basak S, Duttaroy AK: Leptin induces tube formation in first- trimester extravillous trophoblast cells. Eur J Obstet Gynaecol Reprod Biol.164:24-9,2012.

7 Bhaskarachary K, Ramulu, P., Udayasekhararao P, Bapurao S, Kamala, K., Syed, Q., Udaykumar, P, Sesikeran, B: Chemical composition, nutritional and toxicological evaluation of rice (Oryza sativa) grown in fly ash amended soils. *J. Sci. Food Agric. 92:2721-2726,2012.*

8 Bhavesh Sojitra, Yogesh B, Uday Kumar P, Abhinav Kanwal, Prachi Gupta, Madhusudana K, Sanjay Kumar Banerjee: Nitric oxide synthase inhibition abrogates hydrogen sulphide-induced cardioprotection in mice. Mol Cell Biochem. 360:61-9,2012.

9 Bodiga VL Bodiga S, Surampudi S, Boindala S, Putcha U, Nagalla B, Subramaniam K, Manchala R: Effect of vitamin supplementation on cisplatin-induced intestinal epithelial cell apoptosis in Wistar/NIN rats. Nutrition. 28:572-80,2012.

10 Cukras C,Gaasterland T, Lee P,Gudiseva HV, Chavali VR, Raghu P, Maranhao B, Edsall L, Soares S, Reddy GB, Sleving PA,, Ayyagari R: Exome analysis identified a novel mutation in the RBP4 gene in a consanguineous pedigree with retinal dystrophy and developmental abnormalities. PLOS one.7.issue 11.e50205,2012.

11 Chalamaiah M Dinesh kumar B, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant acivities and applications:A review. Food Chem. 135:2020-38,2012.

12 Chandrasekhar Akileshwari, Muthenna P, Branislav Nastasijevic, Gordana Joksic, Mark Peterash J, Bhanuprakash Reddy G: Inhibition of aldose reductase by gentiana lutea extracts. Exp Diabetes Res.ID 147965,2012.

13 Deepika MLN, Ranjith Reddy K,Usha Rani V, Balakrishna N, Prasanna Latha K,Parveen Jahan : Do ACE I/D gene polymorphism serve as a predictive marker for age at onset in PCOS? J Assist Reprod Genet. 30:125-130,2012.

14 Devindra S, Rao J Srinivasa, Bhaskar V, Mulimani VH : Effect of heat treatment on alpha-galactoside content of red gram seeds (Cajanus Cajan L). J Food Process Preserv., 2012.

15 Duttaroy AK, Basak S: Docosahexaenoic acid and angiogenesis: a role in early placentation. Clin Lipidol. 7:303-312,2012.

16 Harishankar N, Seshadri E, Kalyanasundaram S, Giridharan NV: Impaired glucose tolerance (IGT) to frank diabetes: Dietary manipulations in WNIN/GR-Ob rats. J Diabetes Mellit.2:52-58,2012.

17 Hemalatha R, Karthik M, Narendra Babu K, Dinesh Kumar B: Immunomodulatory activity of triticum aestivum and its effects on Th1/Th2 cytokines and NFKB p65 response. Am J Biochem Mol Biol. 2:19-29,2012.

18 Hemalatha R, Mastromarino P, Ramalaxmi BA, Balakrishna NV, Sesikeran B: Effectiveness of vaginal tablets containing lactobacilli versus pH tablets on vaginal health and inflammatory cytokines: a randomized, double-blind study. Eur J Clin Microbial Infect Dis. 31:3097-3105,2012.

19 Hemalatha R, Ramalaxmi BA, Krishna Swetha G, Madhusudhan Rao D, Sesha Charyuly, Dinesh Kumar B: Nutritional status, bacterial vaginosis and cervical colonization in women living in an urban slum in India. Int J Nutr Metab. 4:77-82,2012.

20 Hemalatha R,Ramalakshmi BA,Krishna Swetha G, Uday Kumar P, Madhusudhan Rao D, Balakrishna N, Annapurna V: Cervicovaginal inflammatory cytokines and sphingomyelinase in women with and without bacterial vaginosis. Am J Med Sci. 344:35-9,2012,

21 Johnson W, Shahnaz Vazir, Sylvia Fernandez-Rao, Vijaya R Kankipati, Balakrishna,N, Paula L Griffiths: Using the WHO 2006 child growth standard to assess the growth and nutritional status of rural south Indian infants. Ann Hum Biol. 39:91-101, 2012.

22 Jonnalagadda PR, Jahan P, Venkatasubramanian S, Khan IA, Prasad AYE, Reddy KA, Rao MV, Venkaiah K, Hasan Q: Genotoxicity in agricultural farmers from Guntur district of South India- A case study. Hum Exp Toxicol. 31:741-747,2012.

23 Khalid Hussain Bhat, Krishna Chaitanya C, Nazia Parveen, Raja Varman, Sudip Ghosh, Sangita Mukhopadhyay: Proline-Proline-Glutamic Acid (PPE) protein Rv1168c of mycobacterium tuberculosis augments transcription from HIV-1 long terminal repeat promoter. Journal of Biological Chemistry.287:16930-16946, 2012.

24 Koletzko B, Bhutta ZA, Cai W, Cruchet S, Guindi ME, Fuchs GJ, Goddard EA, van Goudoever JB, Quak SH, Bharati Kulkarni , Makrides M, Ribeiro H, Walker A: Compositional requirements of follow-up formula for use in Infancy: Recommendations of an International Expert Group Coordinated by the Early Nutrition Academy. Ann Nutr Metab.62:44-54,2012.

25 Kumar S, Ali MY, Sailaja P, Mahesh S, Surekha MV, Giridharan NV, Harishankar N: Therapeutic properties of Mucuna pruriens Linn. – an unani drug, in a prediaabetic obese rat model. Int J Body Composition Research.10:1-8, 2012.

26 Kumari M, Rajak S, Singh SP, Kumari SI, Uday Kumar P, Murty US, Hahhoob M, Grover P, Rahman MF: Repeated oral dose toxicity of iron oxide nanoparticles:biochemical and histopthological alterations in different tissues of rats. J Nanosci Nanotehnol. 12: 2149-59, 2012.

27 Laxmaiah A, Nair KM, Arlappa N, Raghu P, Balakrishna N, Mallikharjuna Rao K, Gal Reddy CH, Sharad Kumar, Ravindranath M, Vikas Rao V, Brahmam GNV: Prevalence of ocular signs and subclinical vitamin A deficiency and its determinants among rural pre-school in India. Public Health Nutrition. 15:568-577,2012.

28 Lin Zhang, Sundar Rao K, Jeong SC, Narsimha Reddy, Smith PT, Ananthan R, Longvah T: Antiodidant and immunomodulatory activities of polysaccharides from the roots of Sanguisorba officinalis. Int J Biol Macromol. 51:1057-1062,2012.

29 Little Flower Augustine, Shahnaz Vazir, Sylvia Fernandez Rao, Vishnuvardhana Rao M, Laxmaiah A, Ravinder P, Vikas Rao V , Madhavan Nair K : Psychometric validation of a knowledge questionnaire on micronutrients among adolescents and its relationship to micronutrient status of 15-19–year-old adolescent boys, Hyderabad, India. Public Health Nutrition. 15:1182-1189,2012.

30 Manoj Kumar , Nagpal R, Hemalatha R, Verma V, Kumar A , Tiwari A, , Singh S, Marotta F, Jain S, Yadav H: Targeted Cancer Therapies: The Future of Cancer Treatment. *Acta Biomedica*. 83, 220-233, 2012.

31 Mallikharjuna Rao K, Arlappa N, Radhika MS, Balakrishna N, Laxmaiah A, Brahmam GNV: Correlation of fat mass index and fat-free mass index with percentage body fat and their association with hypertension among urban South Indian adult men and women. Ann Hum Biol. 39:54-58, 2012.

32 Maheshwar M, Raghunatha Rao D: Quantitative analysis of nutrition and health messages in Indian print Media. Public Health Res. 2:28-31, 2012.

33 Mansoori T, Viswanath K, Balakrishna N: Quantification of retinal nerve fiber layer thickness using spectral domain optical coherence tomography in normal Indian population. Indian J Ophthalmol 60:555-8,2012.

34 Meshram II, Arlappa N, Balakrishna N, Laxmaiah A, Mallikharjuna Rao K, Gal Reddy Ch, Ravindranath M , Sharad Kumar S, Brahmam GNV : Prevalence and determinants of under-nutrition and its trends among pre-school tribal children of Maharashtra State, India. J Trop Pedatr. 58:125-132,2012.

35 Meshram II, Arlappa N, Balakrishna N, Rao KM, Laxmaiah A,, Brahmam GNV: Prevalence of hypertension, its correlates and awareness among adult tribal population of Kerala State, India. J Postgrad Med.58:255-61,2012.

36 Meshram II, Arlappa N, Balakrishna N, Rao KM, Laxmaiah A, Brahmam GNV: Trends in the prevalence of undernutrition, nutrient and food intake and predictors of undernutrition among under five year tribal children in India. Asia Pac J Clin Nutr. 21:568-576,2012.

37 Meshram II, Laxmaiah A, Venkkaiah V, Brahmam GNV: Impact of feeding and breastfeeding practices on the nutritional status of infants in a district of Andhra Pradesh, India. Natl Med J India. 25:201-206,2012.

38 Misra S,. Ramesh R, Sita Ramu CH,,Srirangalaxmi G,, Radhakrishna H, Vajreswari A: Case Report: Adrenomyeloneuropathy. J Assoc Physicians India. 60,58-61, 2012.

39 Mrudula T, Prashanth A, Vajreswari A, Giridharan NV, Radha A, Bhanuprakash Reddy G: Vitamin A supplementation ameliorates obesity-associated retinal degeneration in WNIN/Ob rats. Nutrition. 29:298-304,2012.

40 Munshi A, Babu MS, Kaul S, Rajeshwar K, Balakrishna N, Jyothy A: Association of LPL gene variant and LDL, HDL, VLDL cholesterol and triglyceride levels with ischemic stroke and its subtypes. J Neurol Sci. 318:51-4,2012.

41 Muthenna P, Akileshwari C, Bhanuprakash Reddy G: Ellagic acid: a new antiglycating agent: its inhibition of N-(carboxymethyl) lysine. Biochem J. 442::221-230, 2012.

42. Muthenna P, Akileshwari C, Megha Saraswat, Bhanuprakash Reddy G: Inhibition of advanced glycation end- product formation on eye lens protein by rutin. Br J Nutr. 107: 941-949, 2012.

43 Muthenna P, Jessica Ponder, Suryanarayana P, Bhanuprakash Reddy G, Mark Petrash J, LaBarbera DV: The Isolation and characterization of Beta-Glucogallin as a novel aldose reductase inhibitor from Emblica officinalis. PLoS ONE. 7 Issue 4, e31399.

44 Nirmala K, Panpatil VV, Rajkumar Kaleb, Bhaskar V, Kalpagam Polasa: Dose-dependent effect in the inhibition of oxidative stress and anticlastogenic potential of ginger in STZ induced diabetic rats. Food Chemistry 135:2954-2959,2012.

45 Raghu P, Ravindernath P, Sudip Ghosh, Bhanuprakash. Reddy G: Contrasting effects of type 2 and 1 diabetes on plasma RBP4 levels: The significance of transthyretin. *IUBMB Life*. 64: 975-82,2012.

46 Rajender Rao K., Padmavathi IJN, Raghunath M: Maternal micronutrient restriction programs the body adiposity, adipocyte function and lipid metabolism in offspring – a review. Rev Endocr Metab Disord..13:103-108,2012.

47 Rajender Rao K., Padmavathi IJN., Venu L, Raghunath M : Does 11β-HSD1 associate with the development of visceral adiposity in maternal Mg restricted wistar/NIN rat offspring? Endoc. Metabol Syndr.S7:1-3,2012.

48 Rajeshwar K, Kaul S, Al-Hazzani A, Sai Babu M, Balakrishna N, Vandana Sharma , Jyothy A, Anjana Munshi C: Reactive Protein and nitric oxide levels in Ischemic Stroke and Its subtypes: Correlation with clinical outcome. Inflammation. 35:,978-84,2012.

49 Ramasubba Rao V, Muthenna P, Shankaraiah G, Chandrasekhar Akileshwari, Hari Babu K, Suresh G, Suresh Babu K, Sateesh Chandra Kumar R, Rajendra Prasad J, Ashok Yadav P, Mark Petrash J, Bhanuprakash Reddy G, Madhusudana Rao J: Synthesis and biological evaluation of new piplartine analogues as potent aldose reductase inhibitors (ARIs). Eur J Med Chem. 57: 344-361,2012.

50 Satish Sati, Vinay Sing Tanwar, ,Anand Kumar K , Ashok Patowary, Vaibhav Jain , Sourav Ghosh, Shadab Ahmed, Meghana Singh, Umakar Reddy S, Girija Ratna Chandak, Raghunath M, Sridhar S, Chakraborty K, Vinod Scaria , Sengupta S: High resolution methylome map of rat indicates role of intragenic DNA methylation in identification of coding region. PLoS One 7 : e31621, 2012.

51 Satya Prakash M, Benegal V, Sugunan AP, Jeemon P, Balakrishna N, Thennarasu K, Pandian D, Pesala KS: Tobacco use and nicotine dependency in a cross-sectional representative sample of 18,018 individuals in Andaman and Nicobar Islands, India. BMC Public Health.12:515-26, 2012.

52 Sinha JK, Shampa Ghosh, Raghunath M: The neuroscience global village – young investigator programme for students from countries with limited resources on the occasion of the 8th IBRO world congress. Bioessays 34:7-9,2012.

53 Sinha SN, Shivgotra VK: Environmental monitoring of adulterated gasoline with kerosene and their assessment at exhaust level. J Environ. Biol. Vol.33:729-734, 2012.

54 Sinha SN, Vasudev K, Vishnu Vardhana Rao M: Quantification of organophosphate insecticides and herbicides in vegetable samples using the “Quick Easy Cheap Effective Rugged and Safe” (QuEChERS) method and a high-performance liquid chromatography-electrospray ionisation-mass spectrometry (LC-MS/MS) technique. Food Chem. 132:1574-1584, 2012.

55 Sinha SN, Vishnuvardhana Rao M, Vasudev K: Distribution of pesticides in different commonly used vegetables from Hyderabad, India. Food Res Int. 45:161-169, 2012.

56 Sinha SN, Vishnuvardhana Rao M, Vasudev K, Martins Odetokun : A liquid chromatography mass spectrometry-based method to measure organophosphorous insecticide, herbicide and non-organophosphorous pesticide in grape and apple samples. Food Control. 25 (2):636-646, 2012.

57 Sireesha R, Bhagya Laxmi SG, Mamata M, Yadagiri Reddy P, Upender Goud P, Venkateshwar Rao P, Bhanuprakash Reddy G, Vishnupriya S, Padma T : Total activity of glutathione-S-transferase (GST) and polymorphisms of GSTM1 and GSTT1 genes conferring risk for the development of age related cataracts. Exp Eye Res.98:67-74, 2012.

58 Siva Sankara Vara Prasad S , Mahesh S, Vijay Kumar P, Harishankar N, Uday Kumar P, Shailaja P, Swarupa Rani K, Lakshmi Raj Kumar P, Vani Acharya Giridharan NV, Vajreswari A. Carbenoxolone treatment ameliorated metabolic syndrome in WNIN/Ob Obese rats, but induced severe fat loss and glucose intolerance in lean rats. PLOS One 7:e50216,2012.

59 Soundarya LM, Satya S Challa, Maniprabha C, Giridharan NV, Ramesh R Bhonde, Rajanna

A, Vijayalakshmi V: Promise(s) of mesenchymal stem cells as an in vitro model system to depict pre-diabetic/diabetic milieu in WNIN/GR-Ob mutant rats. PLoS ONE. 7:.e48061,2012.

60 Srimanjari K, Nagalla B, Abbagani V, Porika SK, Akka J, Nallari P, Ananthapur V: Role of proteases and antiprotease in the etiology of chronic pancreatitis. Saudi J Gastroenterol 18:364-368, 2012

61 Subba Rao GM: Knowledge sharing and knowledge translation-new perspectives in communication for dietetics. J Ind Dietet Assoc..36s:36-37,2012.

62 Sudershan Rao V, Naveen Kumar R, Kalpagam Polasa: Foodborne diseases in India- a review. Br Food J. 114:661-680, 2012

63 Sudershan Rao V, Naveen Kumar R, Kashinath L, Bhaskar V, Polasa K: Microbiological hazard identification and exposure assessment of poultry products sold in various localities of Hyderabad, India. The Scientific World journal. Article ID 736040, 2012.

64 Suryanaryana Murty U,, Srinivasa Rao M, Madhusuhan Rao K,, Sriram Kumaraswamy, Balakrishna N: A Cohort study of lymphatic filariasis on socio economic conditions in Andhra Pradesh, India." PLOS One 7:e33779, 2012.

65 Taylor AE, Kuper H, Varma RD, Wells JC, Bell JD, V Radhakrishna K, Bharati Kulkarni, Sanjay K, Timpson NJ, Ebrahim S, Smith GD, Ben-Shlomo Y. Validation of dual energy x-ray absorptiometry measures of abdominal fat by comparison with magnetic resonance imaging in an Indian population. PLoS One.7:e51042,2012.

66 Vasanthi S, Uday Kumar, P. Raghu,P., Vishnu Vardhana Rao M., B. Sesikeran, G.S. Ottawa, Priyanka Gupta, Spriha Rao, K. Satyanarayana, V.M. Katoch, T.S. Bharaj, G.S. Mangat, Neerja Sharma, J.S. Sandhu, V.K. Bhargav, Shobha Rank: Aflatoxin contamination in stored rice variety PAU 201 collected from Punjab, India. Indian J Med Res 136: 89-97,2012.

67 Vasuprada Iyengar, Raghu P, Madhavan Nair K: Co-ordinate expression and localization of iron, zinc transporters explains iron-zinc interactions during uptake in Caco-2 cells: implications for iron uptake at the enterocyte. J Nutr Biochem.23:1146-1154,, 2012.

68 Venkatesan V, Chalsani M, Nawaz SS, Bhonde RR, Challa SS, Giridharan NV : Optimization of condition(s) towards establishment of primary islet cell cultures from WNIN/Ob mutant rat. Cytotechnology. 64:139-144, 2012.

69 Vijayalakshmi B, Sreedhar B, Sreedhar S, Sesikeran B, Udaykumar P, Balakrishna N, Kalyanasundaram S, Raghunath M: Effect of vitamin supplementation on cisplatin-induced intestina epithelial cell apoptosis in Wistar/NIN rats. Nutrition.. 28:572-580,2012.

70 Vijayendra Chary A, Hemalatha R, Narendra Babu K, Ramesh kumar R, Sivaram Prasad, K, Dinesh Kumar B: CD23, TH1/TH2 cytokines in bronchial asthma, bronchiolitis and bronchial pneumonia in pediatric patients. Asian J Pharm Clin Res.5:111-114,2012.

71 Vishnuvardhana Rao M, Sharad Kumar, Brahmam GNV: A Study of the Geographic clustering of the state of Uttar Pradesh (UP) in India using nutritional anthropometric data of pre-school children. Indian J Med Res.137:73-81,2012.

72 Yadagiri Reddy P, Giridharan NV, Bhanuprakash Reddy G: Activation of sorbitol pathway in metabolic syndrome and increased susceptibility to cataract in Wistar-Obese rats. Mol Vis 18:495-503, 2012.

73 Yasmeen R, Jeyakumar SM, Reichert B, Yang F, Ziouzenkova O : The contribution of vitamin A to autocrine regulation of fat depots. Biochim Biophys Acta.. 1821:190-197, 2012.

74 Yuncang Li, Cuie Wen, Dolly Mushahary, Sravanthi R, Harishankar N, Gopal Pande, Peter Hodgson: Mg-Zr-Sr alloys as biodegradable implant materials. Acta Biomater.. 8:3177-3188,2012.

B. PAPERS PUBLISHED IN PROCEEDINGS/ BOOKS/CONFERENCES/ABSTRACTS

1 Agath Betsy: Development of food frequency interview schedule for assessing the dietary patterns of vpopulation of Hyderbad" International Conference of Food Studies: An Interdisciplinary Conference held at University of Illinois, Champaign, Illnois on 4-5th October, 2012.

2 Arlappa N, Laxmaiah A, Balakrishna N, Hari Kumar R, Mallikarjuna Rao K, Galreddy Ch, Sharad Kumar, Ravindranath M, Brahmam GNV. Prevalence of Anaemia among rural pre-school Children in India.2nd International conference on Paediatrics and Gynecology".Sept 24-26, 2012.Hyderabad, India.

3 Arlappa N. Epidemiological Review of Preventable blindness in India-A Focus on Vitamin A Deficiency among pre-school children in India: 9th General Assembly of the International Association for Prevention of Blindness (IAPB). Sept 17-20, 2012.Hyderabad, India.

4 Arlappa N, Laxmaiah A, Balakrishna N, Hari Kumar R, Mallikarjuna Rao K, Galreddy Ch, Sharad Kumar, Ravindranath M, Brahmam GNV. "Consumption pattern of pulses and legumes among tribal population in India." At "VI International Conference on Legume Genetics and Genomics" .ICRISAT, Hyderabad. 2-7 October, 2012.

5 Ayesha Ismail, Mehrajuddin Bhat: Vitamin D deficiency induced muscle protein degradation occurs through the ubiquitin proteasome pathway. 15th Vitamin D Workshop, June 20th – 22nd, 2012, Houston, TX, USA.

6 Bhanuprakash Reddy G, P. Muthenna, G. Raghu, C. Akileshwari, P. Anil Kumar P. Suryanaryana P: Antiglycating potential of procyanidin-B2 isolated from cinnamon bark: Prevention of diabetic nephropathy. Annual Conference of Indian Society of Nephrology, Ahmedabad, December 7-9, 2012.

7 Bhanuprakash Reddy G, P Y. Reddy, N. V. Giridharan. Metabolic Syndrome and Cataract: Studies With A Novel Obese Rat Model. *Invest Ophthalmol Vis Sci* 2012;53: Abstract 3041.

8 Bhanuprakash Reddy G. Isolation and characterization of novel aldose reductase inhibitors and antiglycating agents from natural sources: Prospects for alleviating diabetic complications".National Seminar on "Drug Discovery from Plants: Promises & Challenges" organised by School of Life Sciences, SRTM University, Nanded, February 14-15, 2012.

9 Bharati Kulkarni, Juper H, Radhakrishna KV, Hills AP, Byme NM, Ben-Shlomo Y, Davey Smith G, Ebrahim S, Kinra S: Early life nutrition and lean body mass development in young Indian adults. International conference on Nutrition and Growth, Paris, France, Mar.1-3,2012.

10 Bhaskarachary K: Dietetic Profession - Challenges & Opportunities J I D A , Vol 36S,23,2012.

11 Naveena N, Bhaskarachary K: Effect of different drying methods and storage on the content of polyophenols in selected green leafy vegetables. J Ind Diatet Assoc,. 36 S,182,2012.

12 Bindu Noolu, Rajanna Ajumeera, Ayesha Ismail: Murraya koenigii leaf extract induces growth arrest and leads to apoptotic cell death in breast cancer cells. 81st Annual Conference of Society of Biological Chemists India (SBC-I), 8-11th November 2012, Kolkata. India.

13 Chennaiah, Radhakrishna KV, Venkaiah, K, Suresh C, Raghunath M: Effect of supplementation of single vitamin D dose on the serum levels of vitamin D and calcium in the above 50 age groups. Poster communication at 15th workshop on Vitamin D, 20-22 June, Houston, Texas, USA. Special issue, Journal of Steroid Biocheistry and Molecular Biology. 2012.

14 Feary J, Henderson J, Taylor A, Kinra S, Ebrahim S, Krishna K, Ben-Shlomo Y, Kuper H: The Effect of nutritional supplementation during pregnancy and early life on lung function development: follow up of a birth cohort study. Am J Respir Crit Care Med . 185: Abst.p125,2012.

15 Feary J, Henderson J, Taylor A, Kinra S, Ebrahim S, Krishna K, Ben-Shlomo Y, Kuper H: Risk factors for development of chronic obstructive pulmonary disease in a cross-sectional study from South India. Am J Respir Crit Care Med. 185:Abst.p6024,2012.

16 Harishankar N, Kalakumar B, Qadri SSYH, Surya Singh, Khandare AL: Whole Body composition, muscle and nerve conduction velocity of goat kids supplemented with different protein varieties of Lathyrus sativus Linn, International Conference on recent trends in Lathyrus sativus research. NIN,Hyderabad, Nov.8-9, p.33, 2012.

17 Jeyakumar SM, O Ziouzenkova, A Vajreswari. Vitamin A - Key regulator of adipose tissue development and insulin sensitivity: Insight from experimental models. 80th Annual Conference of Society of Biological Chemists India (SBC-I), 12-15th November 2012, CIMAP, Lucknow.

18 Jeyakumar SM, A Sheril, P Uday Kumar, Giridharan NV, A Vajreswari Vitamin A improves hyperglycemia and glucose tolerance but aggravates hepatic steatosis in glucose-intolerant obese rats of WNIN/GR-Ob strain. 43rd Annual Conference of Nutrition Society of India. 11-12 November 2012, NIN,Hyderabad.

19 Kala Kumar B, Singh SN, Qadri SSYH, Harishankar N, Khandare AL: Pre-weanling kids as animal model of neurolathyrism, International Conference on recent trends in Lathyrus sativus research,org. NIN,Hyderabad, Nov.8-9,2012, p.32.

20 Khandare AL, Babu JJ, Shanker Rao, Amol Shirfule: Consumption of lathyrus sativus and lathyrism cases: a recent study in Gondia District, International Conference on recent trends in Lathyrus sativus research,org. NIN,Hyderabad, Nov.8-9, p.30, 2012.

21 Khandare AL, Geddam Babu, Shankar Rao : Villages. In conclusion, the effect of high fluoride water was less than observed elsewhere at this concentration, apparently owing to better nutritional status in the fluorotic village.(Abstract) XXXth Conference of the Internatonal society, Szczecin, Poland, Floride Research, Sep5-8,2012.

22 Kumar M, Nagpal R, Kumar, R Hemalatha R, Tiwari A, PallaviM,Verma V, Kumar A. Role of Probiotic In Chemoprevention of Colorectal Cancer 2011. In: Nagpal, R, Kumar A and Singh R (Eds) Microbes in the Service of Mankind: Tiny Bugs with Huge Impact. I.K. International Publisher, Delhi, India (Accepted 2012). IF 1.528

23 Kumar M, R Kumar , Nagpal R , Hemalatha R, Verma V, Kumar A. Clinical Indications for Bifidobacterium 2011. In: Nagpal, R, Kumar A and Singh R (Eds) Microbes in the Service of Mankind: Tiny Bugs with Huge Impact. I.K. International Publisher, Delhi, India (Accepted 2012). IF 1.528

24 Kumar, R., Singh, S., Grover, S., Hemalatha, R. and Batish, V.K.:Probiotics for better cardiovascular health. Accepted by IK international publisher, New Delhi for the Book entitled “Microbes in the service of mankind: Tiny bugs with huge impact”. I.K. International Publisher, Delhi , India (In Press) Experimental Diabetes Research. 2012.

25 Little Flower A, Nair KM, Sylvia Fernandez Rao, Vishnu Vardhan Rao, P.Ravinder, N.Balakrishna, A.Laxmaiah, Shahnaz Vazir. Low grade inflammation in stressful life events is not associated with hypo-ferremia among adolescent boys. Proceedings of 44th National Conference of Nutrition Society of India, Tirupathi Chapter, Tirupathi, 16-17 November, 2012 (Abstract No.OSCN-27).

26 Maheshwar M, Raghunatha Rao D, Subba Rao GM, Venkaiah K: Evaluation of nutrition reports based on research studies in leading Indian newspapers, “Global meet of biologists and satellite conference on vctor control and management: present status and future strategies, Dec.26-28,P.246, 2012.

27 Maheshwar M, Subba Rao GM, Venkaiah K, Raghunatha Rao D: Evaluation of nutrition reports based on research studies in leading Indian newspapers. “Tenth conference of Indian

association for social sciences and health org by Centre of social medicine and community health, Jawaharlal Nehru University"New Delhi, Abstract ID:308.

28 Mehrajuddin Bhat, Syed SYH Qadri, Ayesha Ismail: Calcium alone can partially correct the muscle atrophy induced by vitamin D deficiency. 81st Annual Conference of Society of Biological Chemists India (SBC-I), 8-11th November 2012, Kolkata. India.

29 Nabil A.M.Bin Omar, Kalakumar B, Qadri SSYH, Surya Singh, Khandare AL: Metabolism of B-oxalyl-L-a,B-diamionpropionic acid in goats, kids, International Conference on recent trends in Lathyrus sativus research, NIN,Hyderabad, Nov.8-9, p.37, 2012.

30 Nair KM. Anemia in pregnancy, iron, folate and vitamin B12 status. NFI Symposium on Newer health and nutrition challenges; NFI, New Delhi on 23rd November, 2012.

31 Nair KM. RDA of Iron for Indians – A case Study of deriving RDA's for micronutrients. 44th Annual Conference of NSI- Pre conference workshops on Recommended dietary allowances and diet planning - I and Food safety and standards - current regulations – II. Srinivasa Auditorium on 15th November, 2012.

32 Nair KM, Little Flower Augustine : Developmental concerns of iron deficiency anemia among adolescents, Department of Home Science, SVU college of Science, Sri Venkateswara University, Tirupathi, pp 15-17, 2012.

33 Nair KM. Sample size and study design. Planning meeting for a potential impact study of wheat flour fortification. India Flour Fortification Network (IFFN), Taj Palace Hotel, New Delhi on 9th November 2012.

34 Nair KM: Scope of food fortification technologies in controlling micronutrient deficiencies in India, "Recent Trends in Research of Nutrition Science" 22nd -23rd, 2012 Abstract org by Department of Food & Nutrition, West Gengal State Univ., North 24 Parganas.

35 Nair KM. Scope of Iron fortified iodized salt in alleviating iron and iodine deficiency disorders in India Thoothukudi Salt Conference Confederation of Indian Industry (CII), Hotel Raj Residency, Thoothukudi, , Tamil Nadu ,31st October, 2012.

36 Nair KM. Natural sources of micronutrients. CoP 11 Satellite Even on Biodiversity Management for Alleviating poverty and malnutrition. Organized by MSSRF & NIN, Hyderabad, 10th October, 2012.

37 Nair KM. Food fortification is an effective strategy to control micronutrient deficiency: Scientific evidence. Proceedings of International Conference on Bioactive Natural Compounds from Plant Food in Nutrition and Health (ICBPN 2012) at Kottayam, Kerala during 20-22 September, 2012.

38 Nair KM. Role of micronutrients during the first 1000 days of life. Scientific Workshop on "Maternal and Child Nutrition: The First 1000 days" organized by Nestlé Nutrition Institute Medical Education Hall, KMC, Manipal,Mangalore 1st July, 2012.

39 Nair.KM. Bioavailability of micronutrients with special reference to iron.National Symposium on Micronutrients, Advanced Research Centre, Nellore, 16-17 June, 2012.

40 Nair KM. Developmental concerns of iron deficiency anemia among adolescents. UGC SAP National Seminar on Developmental concerns of adolescents, Department of home science, SVU College of Sciences, Sri Venkakteswara Univ, Tirupati, 6-7 March 2012.

41 Nair KM. Scope of food fortification technologies in controlling micronutrient deficiencies in India. UGC Sponsored National Seminar on Recent trends in research of nutrition science. Organized by Dept of Food and Nutrition, Barrackpore Rastraguru Surendranath College, in collaboration with dept of food and nutrition, West Bengal State Univ. 22nd to 23rd February, 2012.

42 Petrash JM, M. Puppala, J. Ponder, P. Suryanarayana, G. B. Reddy. D. V. LaBarbera:

Isolation and Characterization of a Novel Aldose Reductase Inhibitor From Emblica officinalis. *Invest Ophthalmol Vis Sci* 2012;53: Abstract 1055.

43 Priyanka Shankar, Arjun L Khandare: Downregulation of osteopontin and reduced biomechanical properties in low calcium and fluoride treated rats. Proceedings of 44th National Conference of Nutrition Society of India, Tirupati during 16-17 November, 2012 (Abstract no-JAEN-01; PN:67).

44 Priyanka Shankar, Arjun L Khandare. Amelioration of adverse effects of fluoride toxicity on calcium homeostasis in low calcium treated rats. Proceedings of 44th National Conference of Nutrition Society of India, Tirupati during 16-17 November, 2012 (Abstract no-OPEN-19; PN:110-111).

45 Raghunath M, Sreeramulu D: Minerals, trace elements and antioxidants In "API textbook of Medicine" Ed by Munjal YP, Vol.1, 9th ed. pp. 1917-1921, 2012.

46 Ramesh Athe, Vishnuvardhana Rao M; Nair KM: Impact of iron fortified foods on hemoglobin concentration during pregnancy and pregnancy outcomes: a systematic review and meta-analysis."30th Annual National Conference, Indian Society for medical Statistics, 6-8 Oct 2012. Pp.36-37, 2012.

47 Sreenivasrao,J, Longvah,T,.Bhaskarachary K: Effect of different cooking methods on carotenoids and their bioaccessibility in Green leafy Vegetables. Journal of Indian Dietetic association. 36S:186,2012.

48 Shirfule AL, Khandare AL: Molecular understanding of kidney stone management: a focus on molecular ligand-target insight of glycolate oxidate (GOX) inhibitors from a traditional ayurvedie polyherbal formulatin. J Nephral Therapeut. 2(5):p.55. (Int conf. and exhibition on Nephrology & Therapeutics,Hilton Chicago, USA. Aug.20-22, 2012.

49 Shirfule AL, Raju Naik Vankudavath, Khandare AL: Human lonotroc glutamate receptors revealed higher selectivityto B-ODAP compared to its native agonist-glutamate. International Conference on recent trends in Lathyrus sativus research,org. NIN,Hyderabad, Nov.8-9,2012, p.19

50 Sinha JK , Giridharan NV, Raghunath M: Can systemic and central IGF1 and BDNF play a role in decreasing the longevity of WNIN obese mutant rats (Abstract) Intl. J. Dev. Neurosci.

51 Qadri SSYH, Surya Singh, Harishankar N, Kalakumar B, Khandare AL: Haematological and histopatholgogical observations in experimental neurolathyrism in goats, International Conference on recent trends in Lathyrus sativus research, NIN,Hyderabad, Nov.8-9, p.35, 2012.

52 Vahini J, Bhaskarachary, K: Foods promoted for diabetes control-what's the truth? J Ind Diatet Assoc., 36S,55,2012.

C POPULAR ARTICLES

1 Meheshwar M: Diet and health status of mother and child in Andhra Pradesh. YOJAN October, pp>11-12,2012.

2 Subba Rao GM, Vijayapushapm T, Venkaiah K, Pavarala V: Quantitative and qualitative analysis of nutrition and food safety information in schoo science textbooks of India. Heal Edu. J.71:725-735,2012.

3 Surekha Venkata M, Uday Kumar P: Does apoptosis contribute to a relatively better prognosis in medullary carcinoma of breast ? J Dr.NTR Univ. of Health Sci. 1:12-16,2012.

SCIENTIFIC ADVISORY COMMITTEE

NIN/FDTRC

Dr.M.K.Bhan .. *Chairperson*
Secretary
Government of India
Department of Biotechnology
New Delhi – 110 003

Prof.D.N.Rao
Department of Biotechnology
All India Institute of Medical Sciences
New Delhi – 110 029

Prof.C.C.Kartha
Professor of Eminence
Rajiv Gandhi Centre for Biotechnology
Thiruvanthapuram
Kerala

Dr.S.Radhakrishna
E-102, High Rise Apartments
Lower Tank Bund Road
Hyderabad – 500 080

Dr.Kumud Khanna
Director
Institute of Home Economics
New Delhi – 110 016

Dr.Promila Pandhi
Department of Pharmacology
Postgraduate Institute of Medical
Education & Research
Chandigarh – 160 012

Dr.P.Reddanna
Department of Animal Sciences
School of Life Sciences
University of Hyderabad
Gachibowli, Hyderabad

Prof. K.R.Thangappan
Professor & Head
Achutha Menon Centre for Health Science
Studies
Sri Chitra Tirunal Inst. of Medical Sciences &
Technology , Tiruvananthapuram
Trivandrum – 695 011, Kerala

Dr.D.Kanungo
H.No.294, Sector 21-D
Delhi – 121 005

Dr.C.J.Yajnik
Director, Diabetes Unit
KEM Hospital
Rasta Peth, Pune – 411 011

Dr.D.C.S.Reddy
Advisor on HIV AIDS Project
Office of the WHO Representative to India,
New Delhi – 110 011

Dr.Sunita Mittal
Head, Dept. of Obstetrics & Gynaecology
All Indian Institute of Medical Sciences
New Delhi – 110 029

Dr.Ashok B Vaidya
Research Director
Kasturba Health Society Medical Research
Centre, Mumbai

Dr.Rajeshwar Dayal
Prof. Government Medical College
Agra, UP – 282 001

NCLAS

Dr.Sandip K.Basu .. *Chairperson*
Director
National Institute of Immunology
New Delhi – 110 067

Dr.T.S.Rao
Adviser, Department of Biotechnology
Block 2, 8th Floor
CGO Complex, Lodi Road
New Delhi – 110 003

Prof.P.B.Seshagiri
Professor, Department of Molecular
Reproduction, Development of Genetics
Indian Institute of Science,
Bangalore – 560 012

Dr.Anurag Agarwal
Scientist
Lab# 615, Institute of Genomics and Integrative
Biology, Mall Road
Delhi

Dr.Ramakrishna Sistla
Scientist
Pharmacology Division,
Indian Institute of Chemical Technology
Hyderabad – 500 007

Dr.S.D.Kholkute
Director
National Institute of Research in Reproductive Health, Jehangir Merwanji Street
Parel, Mumbai – 400 012

Dr.T.R.Raju
Prof & Head
Dept. of Neurophysiology
NIMHANS, Bangalore

Dr.Mitradas M.Paniker
National Centre for Biological Sciences
Tata Institute of Fundamental Research
GKVK, Bellary Road
Bangalore – 560 065

Dr.Subir Majumdar
National Institute of Immunology
Aruna Asif Ali Marg
New Delhi – 110 067

ICMR Officials

Dr.V.M.Katoch
Secretary to Govt. of India
Department of Health Research
(Ministry of Health & Family Welfare) &
Director General
Indian Council of Medial Research
New Delhi - 110029

Dr. G.S.Toteja
Scientist 'F' & Head
Nutrition
Indian Council of Medical Research
V.Ramalingaswami Bhawan
Ansari Nagar, Post Box. 4911
New Delhi – 110 029

Shri T.S. Jawahar, IAS
Sr. Deputy Director General (Administration)
Indian Council of Medical Research
V.Ramalingaswami Bhawan
Ansari Nagar, Post Box. 4911
New Delhi – 110 029

Smt. Dharitri Panda, ICAS
Sr. Financial Advisor
Indian Council of Medical Research
V.Ramalingaswami Bhawan
Ansari Nagar, Post Box. 4911
New Delhi – 110 029