Modification in the expression of peripheral appetite signals during hypoxia exposure contributes to anorexia

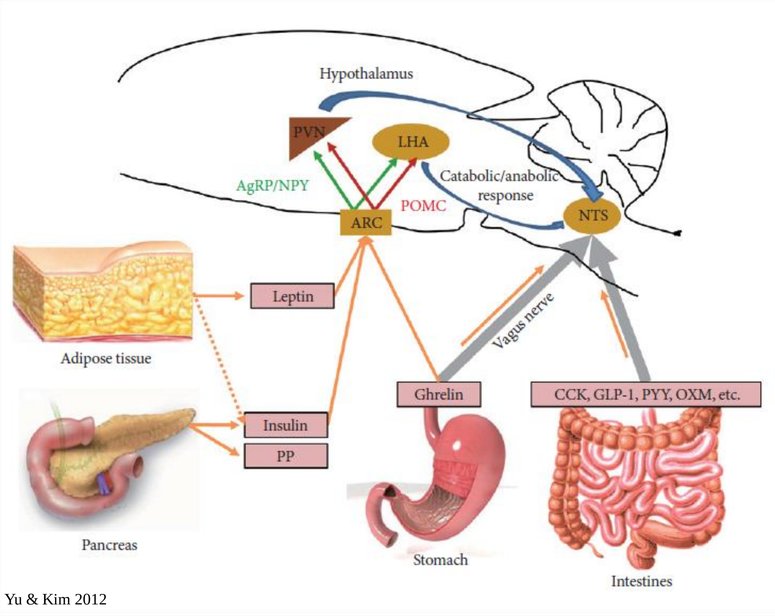
Arul Joseph Duraisamy*, Susovon Bayen, Supriya Saini, Alpesh Kumar Sharma, Praveen Vats and Shashi Bala Singh

Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi – 110054

Email: joeinion@gmail.com

Introduction

- Hypoxia is the main factor at high altitude causing various physiological effects including anorexia.
- Anorexia is one of the major causes for weight loss at high altitude.
- Appetite is regulated by the integration of hormones from gastrointestinal tract, pancreas and adipose in the hypothalamus.
- Appetite regulatory hormones are either orexigenic (feeding inducers) or anorectic (feeding inhibitors).
- ER stress mediated transcriptional and translational alterations caused by hypoxia may alter the synthesis and secretion of hormones that are important in the regulation of feeding behavior during hypoxic exposure.



Yu & Kim 2012

Objectives

1. Screening of appetite regulatory proteins of peripheral origin in rat model of hypobaric hypoxia induced anorexia
2. To find out how cellular expression of appetite regulatory proteins are modulated in adipose tissue
3. To find out appetite related molecular changes in stomach and liver

Materials and Methods

Animals and grouping: Male Sprague Dawley rats weighing 150-200 gms. Six groups with six animals in each group. Groups were named unexposed control, 6 h, 12 h, 1 day, 3 day and 7 day hypoxia exposure.

Hypoxia Exposure: Rats were exposed to hypobaric hypoxia at a simulated altitude of 7,620m in a hypobaric chamber

Body wt. & Food Intake: Every day, body weight and food intake of the rats were monitored while the chamber was opened for replenishing food and water.

Animal sacrifice: All animals were fasted overnight and sacrificed by administering lethal dose of xylazine (10mg/kg body wt.) and ketamine (100 mg/kg body wt.) intraperitonealy. Plasma, epididymal white adipose tissue, liver and stomach tissues were collected for analysis and stored at - 80˚C until use.

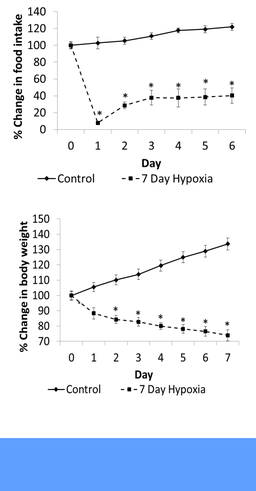
ELISA: Plasma hormones were measured as per kit protocol.

Western blot: Adipose, stomach and liver tissue homogenates were resolved in SDS PAGE, transferred to PVDF membrane and immunobloted for respective primary antibodies

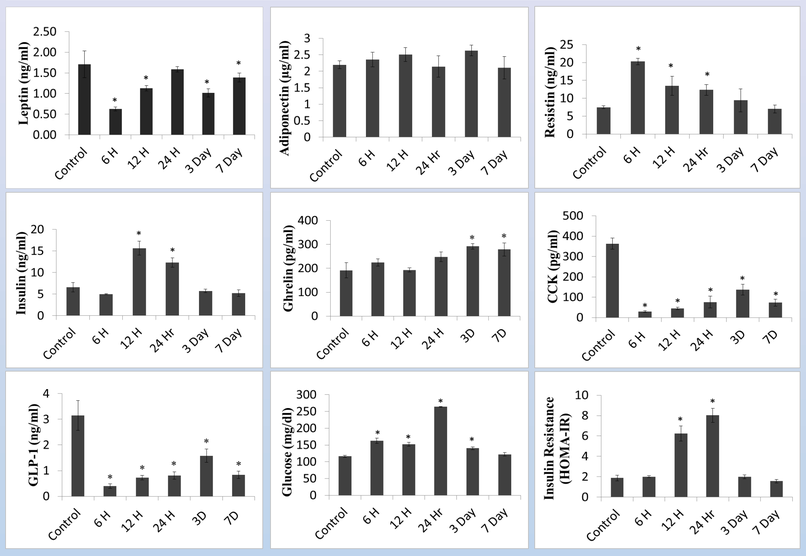
Semiquantitative PCR: Isolated adipose and liver RNA was converted to cDNA using cDNA synthesis kit (Fermentas) and PCR reactions were made using specific primers. Amplicon was resolved in agarose gel and images were captured under UV.

Histology: Formalin fixed sections of adipose tissue were stained with hematoxylin and eosin. Images were captured using Olympus light microscope at 20 X magnification.

Statistics: Data are expressed as mean ± SD. * P<0.05. ImageJ software was used for histology image analysis and densitometry analysis of western blot and PCR images.



Objective 1: Screening of appetite regulatory factors in plasma

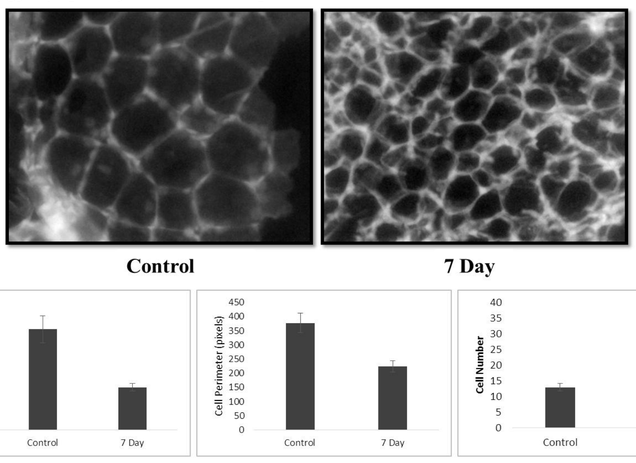


Objective 1: Conclusion

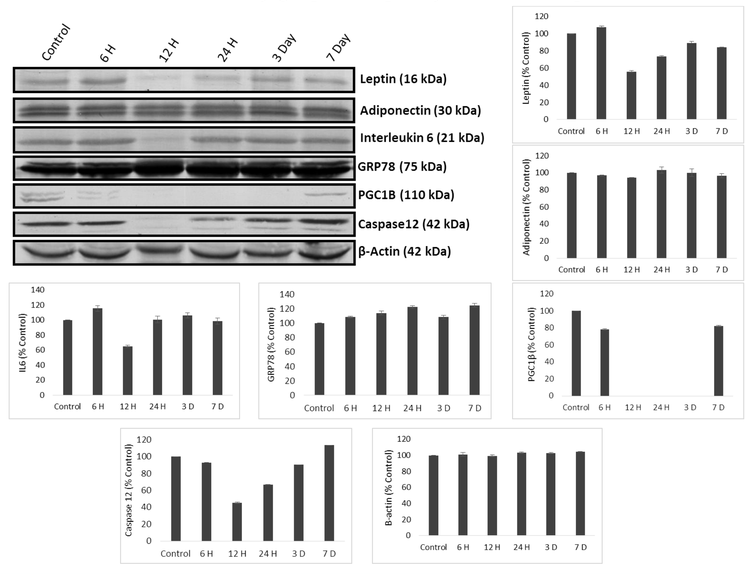
- Adipose derived hormones were modulated during hypoxia
- Gut and pancreas derived hormones behaved properly
- Impairment in glucose clearance

Objective 2: Mechanism of modulation in the expression of appetite regulatory peptides in adipose tissue

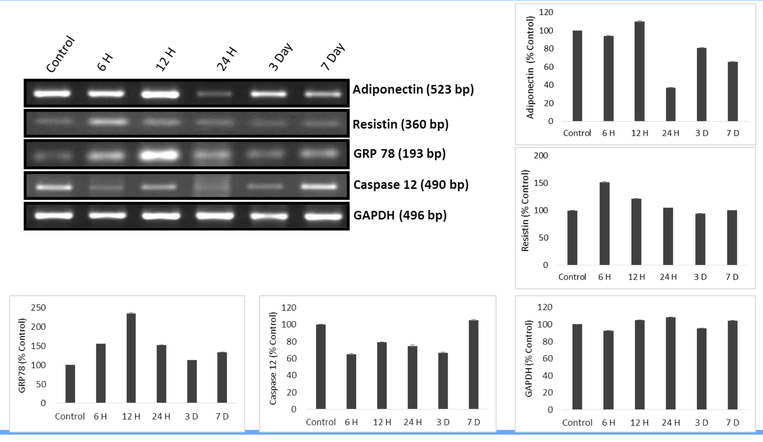
Histology of epididymal white adipose tissue



Western blot and densitometry graphs in epididymal white adipose tissue



Semiquantitative PCR & densitometry graphs in epididymal white adipose tissue

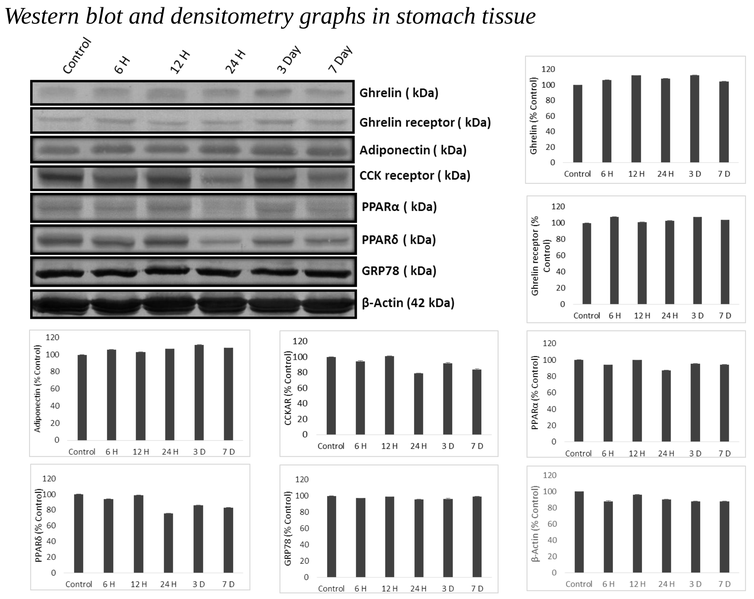


Objective 2: Conclusion

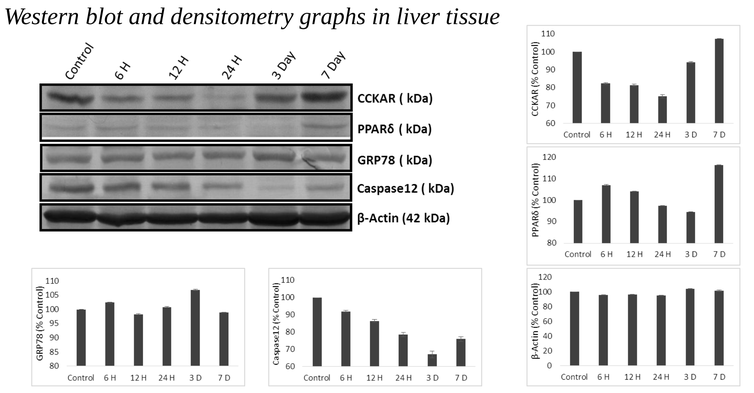
- Loss of fat mass during hypoxia is mainly due to reduction in the adipocyte size
- ER stress occurs during hypoxic exposure in adipose tissue
- ER stress might modulate expression of appetite regulatory proteins in adipose tissue
- PGC1β was reduced in adipose tissue – adipose tissue proliferation halted
- Low caspase 12 expression shows that ER stress did not trigger apoptosis

Objective 3: Appetite related molecular changes in stomach and liver tissue

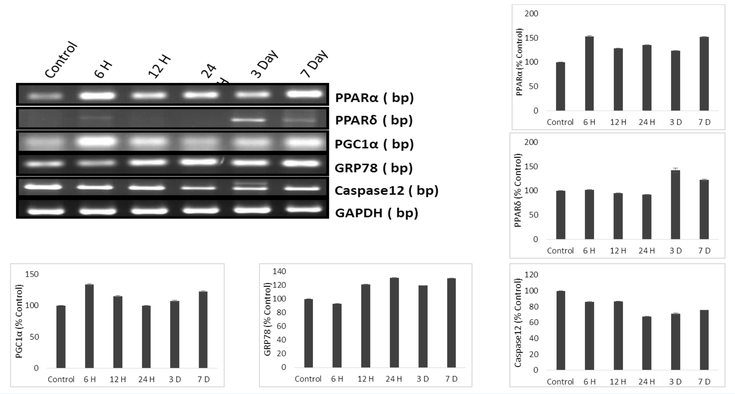
Western blot and densitometry graphs in stomach tissue



Western blot and densitometry graphs in liver tissue



Semiquantitative PCR and densitometry graphs in liver tissue



Discussion

Stomach tissue

- Ghrelin expression increased in stomach.
- Ghrelin receptor levels also increased on exposure and reverted to normal on 7th day.
- Adiponectin which is an adipocyte secretary protein was detected in stomach tissue and found to increase during hypoxia.
- Cholecystokinin receptor level also decreased on hypoxic exposure.
- PPARα and PPARδ were also found to decrease during hypoxia.
- Endoplasmic stress marker GRP78 did not show any change indicating that there is no ER stress in stomach during hypoxia.

Liver

- Cholecystokinin receptor levels decreased during hypoxia and returns to be normal from 3rd day onwards.
- PPARα and PPARδ levels increased in liver tissue during hypoxia as compared to control.
- GRP78 protein and mRNA was observed to increase during hypoxia showing presence of ER stress. Caspase 12 level reduced showing no ER stress mediated apoptosis.

Conclusion

- In conclusion, expression pattern of appetite regulatory hormones were changed during hypoxia. Reduction in adipocyte size was observed which may explain the weight loss. Ghrelin and Cholecystokinin receptors were also modulated in liver which may lead to impairment in glucose metabolism. PPAR isoforms were identified as promising targets in the tissues for alleviating the changes during hypoxia induced anorexia.
- ER stress occurs in adipose and liver but not in stomach tissue indicating a possible tissue specific role of ER stress pathway in response to hypoxia.
- Thus improving food intake and preventing the weight loss by rectifying the changes observed in appetite regulatory hormones and the molecular targets might provide better acclimatization and to cope up with high altitude environment.

Acknowledgment:

Authors are thankful to DRDO for funding the project. Arul Joseph Duraisamy and Susovon Bayen are thankful to DRDO for senior research fellowship. Supriya saini is thankful to UGC for junior research fellowship.