To study chronic hypobaric hypoxia induced metabolic alteration in rat brain using high resolution NMR spectroscopy

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Target: Students, Clinicians and Researchers

Introduction: Low partial pressure of oxygen which prevails at high altitude affects human health adversely. The brain is most sensitive to such hypoxic condition due to its high energy demands[1], particularly hippocampus region, which undergoes neurodegeneration and oxidative stress.[2] It is reasonable to assume that hypoxia induced changes in hippocampus may lead to altered biochemical processes. The dynamic nature of these complex biochemical processes is governed by interconnected regulatory mechanisms[3]. 1H-NMR is a powerful tool to study the metabolic alterations occurring in brain due to high altitude stress. NMR spectroscopy coupled with data reduction and pattern recognition techniques may provide snapshot of biochemical perturbations in response to various stressors. In present study, multivariate technique such as principle component analysis was performed to distinguish between different physiological states occurring in response to high altitude stress.

Objective: Metabolic fingerprinting and biomarker identification of chronic high altitude stress induced changes in hippocampus.

Materials and method: Three months old male Sprague Dawley rats (groups=4, number of animals in each group=6, 250±30g) were taken from animal house, all were fed with a certified standard rat chow and tap water ab libitum. Group 1 was treated as control animals, group 2, 3 and 4 were exposed to altitude of 22,000 feet (temperature 25±1°C and humidity 55±5 %) for 7, 14 and 21 days respectively in climatic hypoxia chamber (Model-SS-7001/ Sevenstar, Delhi, India). All rats were sacrificed and hippocampus was dissected out of brain. The hippocampus extracts were prepared by acetonitrile method[4]. Each lyophilized samples were then mixed with 600µl of D2O consisting of 0.5 mM TSP. 1H NMR spectra were acquired of each sample at 600.09 MHz on a Bruker Avance spectrometer at 298K. 1H-NMR ZGPR experiments (Number of scans=16, locked in D2O, spectral width of 9009 Hz and an acquisition time per scan of 3.64 sec) were performed on samples. Data was further processed with topspin software for base line correction and phase correction, followed by integration of well resolved peaks. Integrated data was statistically analysed with metaboanalyst[5] . The data were normalized by sum, followed by pareto-scaling. One way ANOVA (Turkey's HSD) was applied on normalized data ($p \leq 0.05$).

Results: Clear separation was observed for control group (C) from 7,14 and 21 Days of hypobaric hypoxia in 3D principle component analysis plot (Fig.1). The loading plot (Fig.2) suggested that the hippocampal extracts obtained from hypobaric hypoxia stressed rats contained altered concentration of Lactate, Taurine, Creatine, NAA and Inositol.

Fig-1. PCA plot showing separation of controls (C) from 7, 14 and 21 days of hypobaric hypoxia exposure

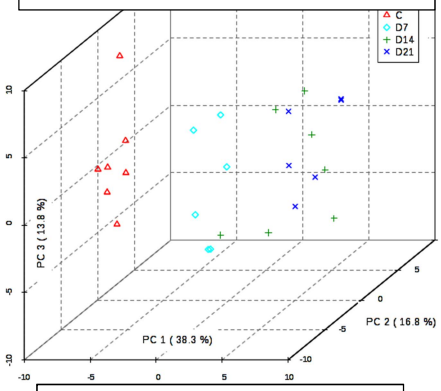

Fig-1. Loading plot showing metabolites responsible for separation in PCA analysis.

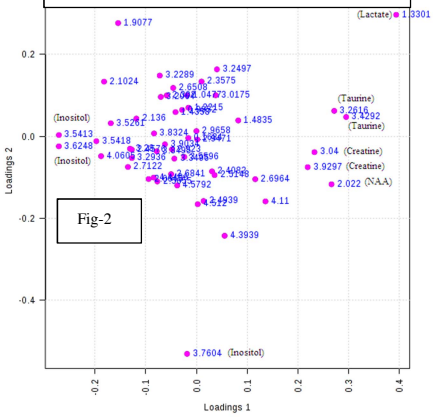

Fig-2

Metabolites	D7 HH	D14 HH	D21 HH
Creatine	--	↑	↑
myo-Inositol	--	↓	↓
Taurine	--	↑	↑
Lactate	↑	↑	↑
NAA	--	↑	↑

Table 1- showing trends of metabolite change in response to hypobaric hypoxia.

Discussion: Creatine may enhance cellularbioenergetics, and acts as a potential neuroprotective agent from the metabolic crisis that ensues during hypoxia. The significant increase in creatine level may indicate enhanced bioenergeticsafter 14 and 21 days of hypobaric hypoxia exposure. Myo-inositol, an osmoregulator may act as a detoxifying substance during severe hypoxic condition. In the present study, myo-inositol level was significantly decreased after 14 and 21 days of hypobaric hypoxia exposure. Taurine apart being an osmolyte has other functions in brain including neuroprotection. Interestingly, present study showed opposite trend with respect to myo-inositol. Lactate may be related to altered bioenergetics of brain cells, which was found increased at all time points. NAA is an important brain metabolite which is neuronal in origin, change in this metabolite may indicate neuronal changes. The significant increase in NAA may result from recovery of neuronal metabolism, and possibly increased dendritic sprouting, synaptogenesis, and neurogenesis.

Conclusion: High resolution 1H-NMR of hippocampal extracts shows a change in complex biochemical processes in response to external stimuli, such as high altitude stress. Results indicate effect of Hypobaric Hypoxia occurring at metabolite level which appears to be due to neuronal changes, altered cellular bioenergetics and change in water diffusion dynamics. Further, these results can be correlated with in vivo and behavioral studies to detect if these alterations have effect on memory functions of brain for risk assessment & early diagnosis.

References:

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