Metabolic Regulatory Variation in rat Serum due to Cold Stress: High Resolution ^{1}H NMR approach

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Introduction: Acute cold stress may trigger systemic biochemical and physiological changes in living organism leading to rapid loss of homeostasis which is reversible process. Prolonged or repeated stress is well recognized as a major risk factor for many disorders including cardiovascular diseases and psychiatric illnesses, such as hypertension, myocardial infarction and depression[1,2]. In severe stressful conditions like cold and hypoxia, higher rate of metabolism is required to cope with increased energy demand[3]. Cold stress results in increased sympathetic activity and activation of hypothalamic-pituitary-adrenal (HPA) axis causing neuro-humoral and metabolic changes for adaptation[4]. These changes can be directly reflected in blood or serum which provides biochemical status of living organism. NMR spectroscopy in conjugation with statistical analysis such as Principal Component Analysis (PCA) can be successfully used as a non-invasive tool to identify biomarkers for an early biochemical changes induced due to cold exposure.

Aim & Objective: To study the changes in metabolic profile & identify potential biomarkers in rat serum due to prolonged cold stress.

Materials & Methods: 8-10 week old male Sprague Dawley rats (233±30g) were housed individually in stainless steel wire mesh cages. Room temperature & humidity were regulated at 24±1ºC & 40±10%, respectively. Rats were acclimatized in metabolic cages & they were divided into four groups, control (C, n=6), cold exposure for 1day (CE1, n=6), cold exposure for 5 days (CE2, n=6) and cold exposure for 15 days (CE3, n=6). On day 0, CE1, CE2 and CE3 groups were exposed to 4ºC for 8 hrs (9a.m. to 5p.m.). Blood sample (~ 1mL) was collected from CE1group after day 1 by heart puncture. CE2 and CE3 groups were exposed to cold stress for 5 and 15days, respectively (4ºC for 8 hrs on each day) and blood samples were collected. Serum samples of 300µl obtained from blood were mixed with 300 µl of D_2O and transferred to 5mm NMR tube with 1mM TSP (in capillary) as an external reference compound for spectral acquisition. ^{1}H NMR spectra were acquired on each sample at 400.13MHz on a Bruker Avance 400 spectrometer at 298K. Water signals and broad protein resonances were suppressed by a combination of presaturation and the CPMG pulse sequence ($\tau = 200\mu s$, n = 500). For each sample, 64 transients were collected into 32K data points with a relaxation delay of 2s, spectral width of 9612 Hz and an acquisition time per scan of 3.98 sec. Peaks or multiplets of identified metabolites, including that of internal standard TSP, were integrated to obtain signal intensity. Multivariate analysis was used i.e. Principle Component Analysis (PCA) to carry out data reduction & by using one way ANOVA, the bin values showing significant difference ($P < 0.05$) were calculated to differentiate control from cold stress groups (CE1, CE2 and CE3).

Results: ^{1}H NMR spectra of serum samples for cold exposed rats (Fig 1) showed a marked continuous increase in metabolites viz lactate, alanine and glucose on prolonged cold exposure for day 1, 5 and 15. However, using one way ANOVA, rest of the metabolites seen in the ^{1}H NMR spectra did not show any significant difference between control, CE1, CE2 and CE3 groups. Also, there was a decrease in total weight of the rats from 230±30g, 214±30g to 209±30g after cold exposure for 1, 5 and 15 days, respectively.

Discussion: Exposure to cold stress of 4ºC at different time point showed perturbations in endogenous metabolites. Visual comparison of ^{1}H NMR spectra (Fig. 1) of serum samples between control and cold exposure for 1, 5 and 15 days (8hrs each day) showed statistically significant increase in energy metabolites such as lactate, alanine and glucose. Increase in lactate concentrations indicate heightened anaerobic carbohydrate metabolism which results from increased energy demands due to cold exposure indicating changes in carbohydrate and energy metabolism[5]. The increased lactate is consistent with incomplete glucose oxidation which might contribute to decreased muscle thermogenesis. There is an increased availability of glucose to muscles as indicated by elevated glucose levels suggesting more carbohydrate metabolism via glycolytic than oxidative pathways during prolonged cold exposure[6]. Increased alanine also suggests a metabolic switch towards energy conservation. Also, a decrease in total weight might be a result of decreased muscle masses which can have a negative impact on total oxidative capacity of muscles. A 3-D PCA analysis (Fig. 2) showed time dependent, clear separation in the first principal component (PC) of the serum profile of rats exposed to cold stress for different time point. With the progression of cold exposure, rats showed higher metabolic changes on day 15 as compared to day 1 and could be easily separated from control indicating systemic metabolic variation due to cold exposure.
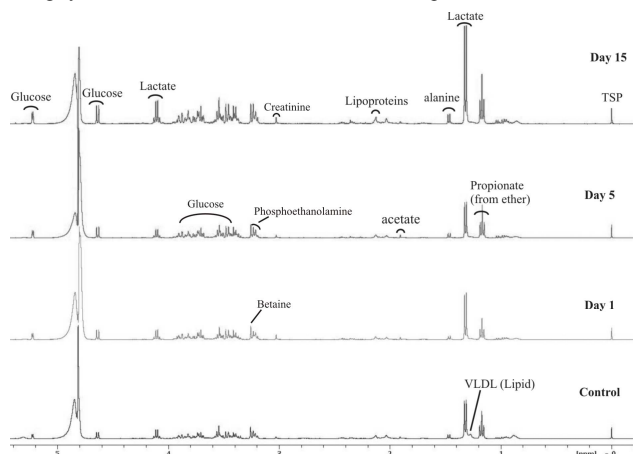

Fig 1: Comparison of ^{1}H NMR spectra of C, CE1 (day 1), CE2 (day 5), CE3 (day 15) group serum samples showing increased intensity of metabolites with increased duration of cold exposure
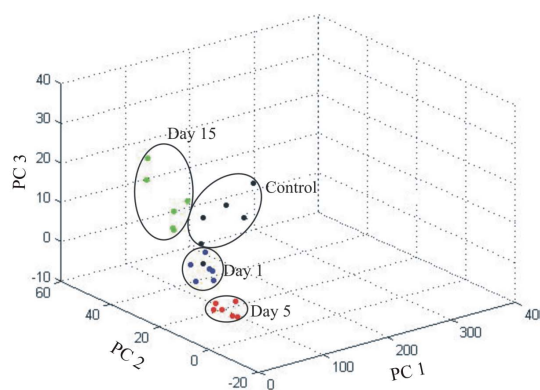

Fig 2: 3D PCA plots for serum samples showing distinct variation between control & cold stress groups (CE1, CE2 & CE3) with increased duration of cold exposure.

Conclusion: In the present study, NMR based metabonomics in conjugation with statistical analysis serves as a powerful tool for non-invasive monitoring of various metabolic alterations in serum induced by prolonged cold stress. These results can be correlated with other biochemical, analytical & molecular parameters to detect early biomarkers for cold stress injuries in humans. This will help in developing strategies to combat variable climatic conditions and mass screening for risk assessment.

References:
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