High Resolution ^{1}H NMR Approach to study the Effects of Cold Stress on the Metabolism of Rat Renal Tissue

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Introduction: Exposure to acute stress conditions is responsible for affecting multiple biochemical regulatory systems and triggering various disorders[1,2]. Acute stress such as extreme cold or heat, panic, toxins, tension may result in development of neuro-psychiatric symptoms such as depression and cognitive impairment[3]. Evidence exist for the adverse effects of acute cold stress on human health including cardiovascular and respiratory diseases like hypertension, asthma, diseases related to immune system and diarrhea[4]. It results in increased sympathetic activity and activation of hypothalamic-pituitary-adrenal (HPA) axis causing neuro-humoral and metabolic changes for adaptation[5]. Acute cold stress may result in immune suppression, gastrointestinal mucosal damage, affects central nervous system (CNS) & potential dysfunction to adrenal glands, kidney & lungs. These changes in metabolic activities of cells/tissues can directly be reflected in biological fluids & can be studied using NMR spectroscopy or LC-MS. However, NMR based tissue metabolic studies after cold exposure are almost negligible in literature.

Aim & Objective: To access the changes in metabolite pattern & identify biomarkers in rat renal tissue due to exposure to cold stress.

Material & 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.). Animals were exsanguinations from cervical dislocation after 1, 5 & 15 days of cold exposure. Kidneys were excised, washed with phosphate buffer and immediately snap frozen in liquid nitrogen. Polar metabolites from kidney tissues were extracted using perchloric acid & supernatant containing metabolites were lyophilized for 10-12 hrs and then dissolved in 600µL of deuterium oxide 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 400.13 MHz on a Bruker Avance 400 spectrometer at 298K. Water suppression was achieved using 1D NOESY pulse sequence. For each sample, 64 transients were collected into 32K data points with a relaxation delay of 2 s, flip angle of 90° and a mixing period of 100 ms. A spectral width of 9612 Hz and an acquisition time per scan of 2.56 s was used. Concentration for each metabolite was calculated[6] by identifying the peaks & integrating wrt TSP. Metabolites concentration was compared in C, CE1, CE2 & CE3 groups using one way ANOVA. Binning of each spectrum was carried out using Chenomx Suit 6.0 & Principle Component Analysis (PCA) was done.

Results: ^{1}H NMR spectra of renal tissue for cold exposed rats showed a marked increase in metabolites viz Lactate, Creatine, Myoinisitol, Glycine & Glucose from day 1 to 5 whereas it decreased on further exposure up to day 15. However, rest of the metabolites seen in the ^{1}H NMR spectra did not show any significant difference between control & cold stress rats (Table 1).

Discussion: Due to cold exposure of 4ºC, ^{1}H NMR spectra of renal tissue samples showed statistically significant increase in metabolites concentration indicating altered biochemical pathways. Visual comparison of ^{1}H NMR spectra of renal tissue samples between control and cold exposure for 1, 5 and 15 days (8hrs each day) showed statistically significant increase and then decrease in energy metabolites such as lactate, Creatine, Myoinositol, Glycine & Glucose. Lactate production is a sensitive and global marker of compound induced cellular stress. Increased lactate production is likely a consequence of converging molecular and functional disturbances, which allow the cell to maintain ATP concentrations through the non-aerobic pathway suggesting increased anaerobic glycolysis[7]. Creatine levels increases and then decreases back to that of normal on exposure to cold stress indicating altered glomerular filtration rate whereas on continuous exposure, the body adapts to the cold stress by maintaining homeostasis and creatine levels starts getting down to normal. Increase in creatine level might be an indicative of damage/swelling of blood vessels, death of cells or reduced blood flow in kidney. It may also increase temporarily as a result of muscle injury. Myoinositol (an osmolyte) is an indicator of osmotic regulation. Increase in inositol levels on cold stress indicates alterations in kidney osmotic balance. When normal glucose regulation fails, the kidneys must remove excess glucose from the blood, leading to high concentrations of glucose in the kidneys. Hence, increase glucose indicates 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 which is consistent with increased lactate concentration. A 3-D PCA analysis (Fig. 1) showed time dependent, clear separation in the first principal component (PC) of the kidney tissue profile of rats exposed to cold stress for different time point. With the progression of cold exposure, rats showed higher metabolic changes on day 5 as compared to day 1 and could be easily separated from control indicating systemic metabolic variation due to cold exposure whereas on further exposure, there is a decrease in metabolite concentration possibly indicating a progression towards adapting to the extreme cold stress.

Metabolites	Control	Cold stress group (after 1 day)	Cold stress group (after 5 days)	Cold stress group (after 15 days)
Valine	0.207±0.037	0.217±0.079	0.196±0.069	0.225±0.063
Lactate	4.493±0.206	6.431±2.151*	11.967±1.57*	4.389±0.991
Alanine	1.558±0.079	1.617±0.370	4.509±1.099	1.260±0.199
Glutamate	3.991±0.333	3.78783±0.605	10.448±1.083	4.0716±0.706
Succinate	2.764±0.274	2.853±0.706	7.221±0.230	2.757±0.674
Creatine	0.656±0.295	2.357±0.601*	2.047±0.783*	1.562±0.490*
Choline	3.633±0.243	4.199±0.735	13.131±4.626	4.110±0.902
TMAO	2.174±0.256	2.858±0.441	9.682±1.254	2.836±0.901
Taurine	4.670±0.626	6.661±1.892	19.070±1.942	5.921±2.305
Myoinisitol	5.634±0.591	8.000±1.450*	19.042±2.613*	6.037±1.511
Glycine	2.460±0.298	3.484±0.714*	7.935±0.979*	2.490±0.762
Sarcosine	2.355±0.152	3.119±0.555	8.590±1.024	2.701±0.695
glucose	0.692±0.169	1.376±0.776*	2.614±0.393*	0.502±0.140

*Significant at 0.05 level

Table 1: The concentration of various metabolites (µmol/g wet weight) from PCA extracts of kidney tissue for control, CE1, CE2 & CE3 after cold exposure.

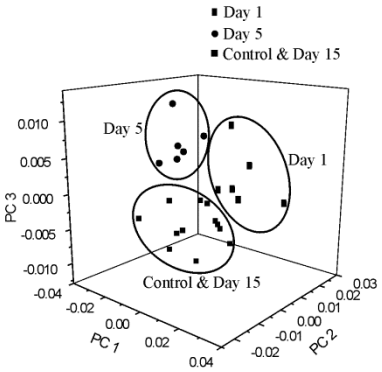

Fig 1: 3D PCA plots for kidney tissue samples showing distinct variation between control & cold stress groups (CE1, CE2) with increased duration of cold exposure whereas with further exposure metabolite levels starts getting back to normal due to homeostasis.

Conclusion: NMR based metabonomics in conjugation with statistical analysis reveals a subtle interplay of functional metabolites and pathways leading to an understanding of systemic response to external stimuli, such as cold stress. The results of this work show significant alterations in low molecular weight metabolite patterns of kidney tissue arising as a result of stress-induced metabolite responses. These results can be correlated with other biochemical, analytical & molecular parameters to detect early biomarkers for cold stress injuries in humans & organ specific dosimetry. Present studies along with NMR studies of urine & serum metabolite changes can give a comprehensive profile of metabolites getting altered due to cold stress.

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