Open Access

Correlation of Age-Related Metabonomic Changes in ^{1}H NMR Serum and Urine Profiles of Rats with Cognitive Function

Abbas Ali Mahdi*[,1], Sanjay Annarao[2], Sandeep Tripathi[1], Akbar Nawab[1], Farzana Mahdi[3], Mahdi Hasan[4], Raja Roy[2] and C.L. Khetrapal[2]

[1]Departments of Biochemistry, King Georges' Medical University, Lucknow-226003; [2]Centre of Biomedical Magnetic Resonance, Sanjay Gandhi Post Graduate Institute of Medical Sciences Campus, Raebareli Road, Lucknow-226014; [3]Department of Biochemistry, Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India; [4]Anatomy, King George's Medical University, Lucknow-226003, India

Abstract: Age related metabonomic changes in young and aged male albino rats have been investigated using Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy. The studies were performed using *'in vivo'* behavioral and metabonomic assessment in serum and urine of the rats. Significant changes ($p<0.05$) were obtained in the concentration of creatinine, allantoin and hippurate in urine. Also significant ($p<0.05$) alterations were also discernible in acetate, pyruvate, lactate, creatine and glucose in serum. Attempts have been made to correlate these changes with the cognitive impairment and perturbed energy metabolism in aged rats. The metabonomic evaluation of age related changes in serum and urine showed altered concentrations of many Krebs cycle intermediates in old rats. Likewise it was associated with diminished mitochondrial functioning. On the basis of our results it is suggested that the observed changes may be an early clinical indication of cognitive decline related to dementia.

INTRODUCTION

Elderly people are known to have the highest incidence of illness and deficits in cognitive functioning [1]. In recent years relatively little effort has been made to investigate the alterations in physiological profiles of this wide group of population. A progressive decline in the efficiency of various physiological processes is perhaps the most noticeable manifestation of ageing along with loss of motor ability and the stamina for sustained physical activity [2-4]. The interplay of biochemical changes, cellular responses and reactions of an organism ultimately lead to the generation of aging phenotypes in which the fitness of the organism is grossly compromised. The phenomena of aging affect cells, tissues and the organism as a whole. The molecular and cellular events involved in aging, especially in a complex multicellular organism, are not clearly understood and this has given rise to a multiplicity of theories which overlap each other to a considerable extent [5]. One of the several explanations put forth to explain the nature of mechanism underlying senescence point towards loss of functional capacity attributable to cellular and molecular damage.

Metabolism is the foundation of all living systems and any biological function is the manifestation of the global cellular metabolism. Metabolites, the end products of cellular processes, reflect the system level biological stress response. Hence, any enzymatic perturbation is directly or indirectly related to the cellular behavior and its metabolism. The metabolites related to metabolism provide a precise snapshot of the system biology. The use of comprehensive ''global metabolite'' profiling methods of analysis for biological fluids and tissues employed in metabonomic studies can provide novel insights into biological processes [6]. Typically, such studies have been performed using ^{1}H NMR spectroscopy. This methodology is now well-established for the evaluation of metabolic perturbations associated with organ specific toxicity [7] or differences between genders, strains and diurnal changes in rodents [8-10]. ^{1}H NMR spectroscopy provides enriched information about low-molecular weight metabolites in body fluids such as serum and urine [11]. ^{1}H NMR spectroscopy has been utilized to obtain detailed information on the concentration of the low-molecular weight metabolites in serum/plasma and urine in a single experiment [12-17].

In this study we explored the relationship between tests of cognitive function and metabolite levels in serum and urine of young (6-months) and old (24-months) male albino rats using ^{1}H-NMR.

MATERIALS AND METHOD

Twenty young male albino rats at 6 month of age (Weight, 200 $\pm$ 20g) and twenty 24 month old rats (weight range, 500 $\pm$ 20g) were obtained from Industrial Toxicology Research Centre, Lucknow, India. They were housed individually in the metabolic cages in a well-ventilated room under controlled conditions (temperature, humidity and a 12 h light-dark cycle). Food and tap water were provided *ad libitum*.

The urine samples were collected from all the rats and centrifuged at 3000 rpm for 5 min at 4^0C to remove the particulate contaminants. On the other hand, blood samples (2 ml) were obtained by venipuncture aseptically. Immediately

*Address correspondence to this author the Professor of Biochemistry, Medical Elementology & Free Radical Biology Lab., Department of Biochemistry, King George's Medical University, Lucknow-226003, India; Mobile: +91-9839011192/9415007706; Tel/Fax: (+91-522) 2253030; E-mail: mahdiaa@rediffmail.com

1874-7698/08 **2008 Bentham Open**

after the collection, the blood samples were placed in a sterile stoppered test tube and were allowed to coagulate for 30 min and centrifuged at 3000 rpm for 5 min at 4^0C to separate the sera. Serum and urine samples were stored at -80^0C until NMR spectroscopic analysis was carried out.

NMR Experiments

^{1}H NMR spectra for all serum and urine samples were obtained on a Bruker Biospin Advance 400 MHz spectrometer (Switzerland) using 5-mm Broad Band Inverse probe head with Z-shielded gradient at 300 K. Serum and urine samples (500 µL each) were taken in 5-mm NMR tubes, a sealed co-axial capillary tube containing 0.375% trimethyl silyl propionic acid sodium salt–d_4 (TSP) in 35 µL deuterium oxide was inserted into the NMR tube before obtaining the NMR spectra. TSP served as a chemical shift reference as well as the standard signal for semi-quantitative estimation of the metabolites, while deuterium oxide served as solvent for 'field-frequency-locking'. One-dimensional ^{1}H NMR spectra were obtained for the samples using one-pulse sequence with suppression of water resonance by presaturation. For all the serum samples, additional one-dimensional ^{1}H NMR spectra were also obtained using Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with suppression of water resonance by presaturation to remove the broad resonances arising from macromolecules. Typical parameters used were: spectral width: 6000 Hz; time domain points: 32K; relaxation delay: 5s; pulse angle: 90°; number of scans: 128; spectrum size: 32 K and line broadening: 0.3 Hz. For CPMG experiment total echo time of 0.64 ms with 150 echoes was used. Semi quantitative concentrations of metabolites were obtained using the integral area of respective metabolite marker signal with reference to the integral area of TSP in CPMG spectra [18].

Behavioral Studies

The spontaneous motor activity (SMA), righting reflex, catalepsy, muscle coordination, active avoidance and passive avoidance were in accordance with a previously described method [19].

Statistical Analysis

The results obtained from quantification of NMR-based metabolites are expressed as Mean ± S.D. The statistical significance for the quantitated metabolites was determined by Mann-Whitney *P* test. Probabilities, *P* less than 0.05 were taken to indicate statistical significance.

RESULTS

Proton NMR Spectral Analysis of Serum of Young and Aged Rats

Typical ^{1}H NMR serum spectra of young and aged rats is shown in Fig. (**1**). Eight metabolites viz., lactate, alanine,

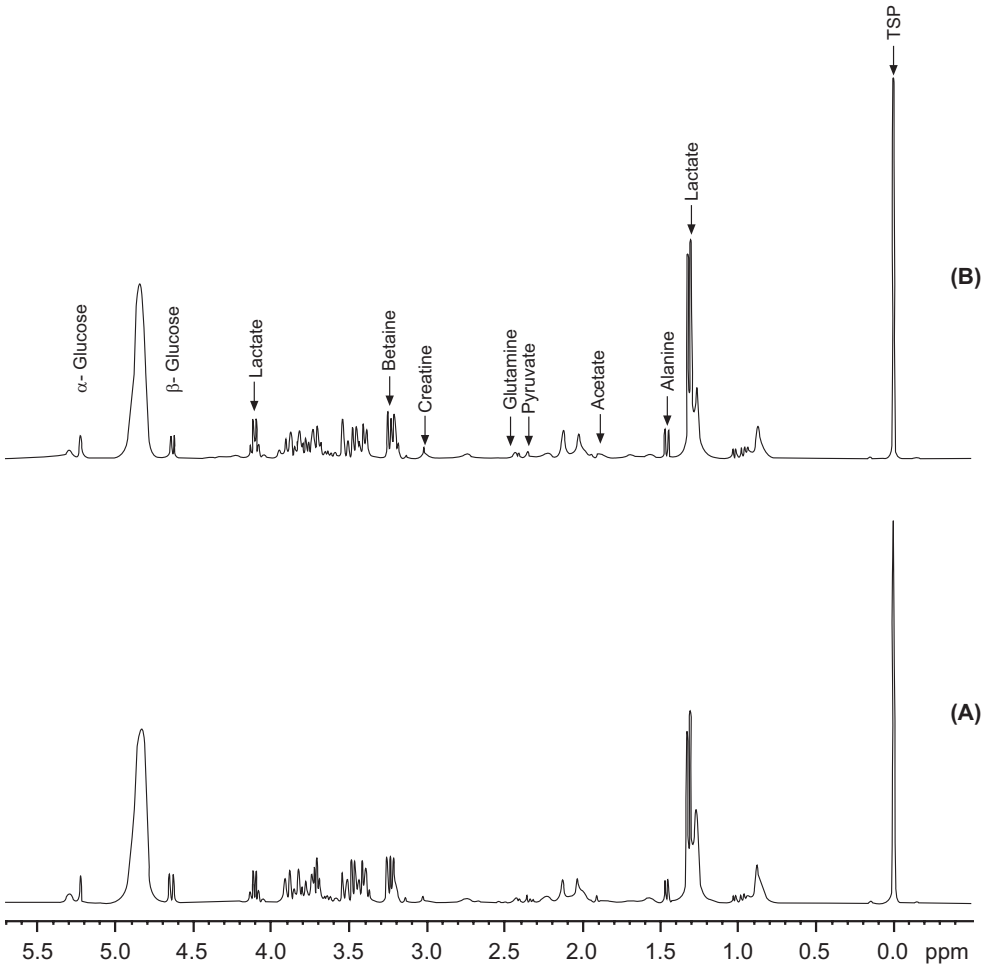

Fig. (1). ^{1}H-NMR serum spectra of young rat (**A**) and aged rat (**B**).

Table 1. Semi Quantitative Concentration of Serum Metabolites in mg/ dL of Young and Old Rats

	Young Rats (n=20) Mean ± SD	Aged Rats (n=20) Mean ± SD	*P*-Value
Alanine	9.4±1.6	8.9±1.7	NS
Acetate	1.1±0.2	0.8±0.2	<0.01
Glutamine	10.3±4.8	9.3±0.9	N.S
Pyruvate	2.2±0.5	1.9±0.5	<0.05
Lactate	72.0±14.1	88.6±24.9	<0.02
Creatine	3.0±1.0	4.5±2.0	<0.01
Betaine	3.8±0.7	4.0±0.6	N.S
Glucose	138.3±29.5	89.5±21.7	<0.01

NS = Not significant.

acetate, pyruvate, glutamine, creatine, betaine and glucose were identified and quantified using their respective ^{1}H NMR signals [20]. The concentration of these eight serum metabolites is shown in Table **1** along with statistical evaluation. Significant reduction was found in acetate, pyruvate and glucose in aged rats as compared with young rats, while, the concentration of lactate and creatine in the young was significantly high than that of aged rats.

Proton NMR Spectral Analysis of Urine of Young and Aged Rats:

Typical ^{1}H NMR spectra of urine of young and aged are shown in Fig. (**2**). Seven metabolites viz., succinate, citrate, α-ketoglutarate, trans-aconitate, creatinine, allantoin, and hippurate were identified and quantified. The concentrations of these urine metabolites are shown in Table **2** along with statistical evaluation. Concentrations of creatinine, allantoin and hippurate were found significantly increased in aged rats as compared with young rats.

Behavioral Studies

Locomotors activity, righting reflex, catalepsy, rota rod, active avoidance and passive avoidance test are presented in Table **3**. Significant changes were found in spontaneous motor activity ($p<0.001$), righting reflex ($p<0.05$), muscle coordination (rota rod) ($p<0.05$) and active avoidance ($p<0.05$) in aged rats as compared with young rats.

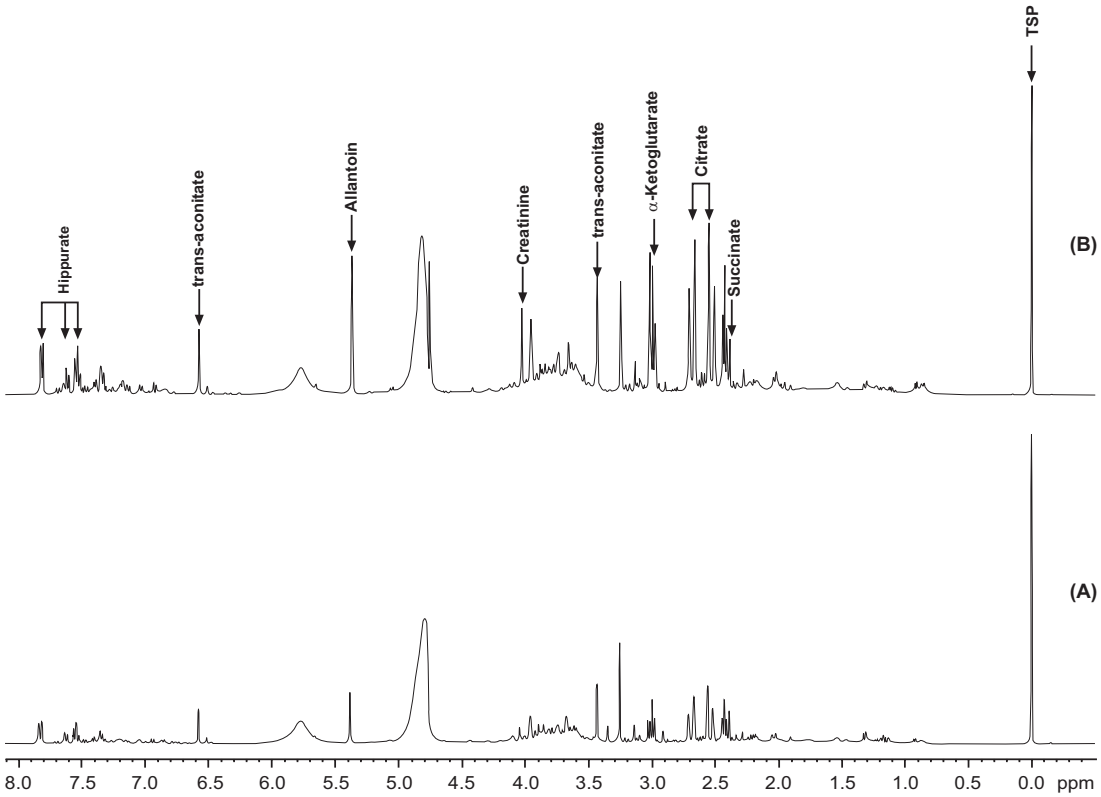

Fig. (2). ^{1}H-NMR urine spectra of young rat (**A**) and aged rat (**B**).

Table 2. Semi Quantitative Concentration of Urine Metabolites in mg/ dL of Young and Old Rats

Metabolites	Young Rats (n=20) Mean ± SD	Aged Rats (n=20) Mean ± SD	*P*-Value
Succinate	62.3±25.4	71.2±38.9	NS
∝-Ketoglutarate	251.1±162.3	316.6±196.6	NS
Citrate	337.5±182.5	457.9±259.3	NS
Creatinine	38.7±18.6	104.6±54.4	<0.01
Allantoin	203.4±94.6	389.5±193.5	<0.01
Trans-Aconitate	60.5±43.6	85.4±49.9	NS
Hippurate	104.4±47.9	250.7±132.6	<0.01

NS = Not significant.

Table 3. Behavioral Profile of Young and Aged Rats

Groups	Young Rats (n=20) Mean ± SD	Aged Rats (n=20) Mean ± SD	*P*-Value
SMA	4.6 ±0.9	3.4 ± 1.0	<0.01
Righting reflex	3.9 ± 1.3	5.3 ± 1.5	<0.01
Catalepsy	12.3 ± 6.2	10.8 ± 4.2	<0.598
Rota Rod	76.8 ± 21.2	61.2 ± 25.7	<0.044
Active Avoidance Test	3.9 ± 1.2	5.2 ± 1.7	<0.014
Passive Avoidance Test	71.9 ± 30.4	90.6 ± 49.8	0.310

DISCUSSION

With advancing age numerous physiological changes take place and they are reflected by the physical and biochemical perturbations in the animal, resulting in a difference in proportion of endogenous metabolites excreted in serum and urine. There have been reports of NMR studies providing evidence that ageing reduces the production of energy *via* oxidative phosphorylation due to interference with many key enzymatic processes of TCA cycle [21]. Moreover, some previous studies in mice have reported age related perturbations in TCA cycle enzymes and impairment in energy pathways [22, 23].

Our NMR data demonstrated that many metabolites associated with the energy producing pathway alter with age. We observed that serum acetate significantly decreased (25%) in aged rats as compared with the young rats. Acetate is the end product of fatty acid oxidation (degradation of short chain fatty acid: acetoacetate + acetyl CoA → acetoacetyl-CoA + acetate). The reduced serum acetate levels may be due to altered ketone bodies metabolism [24]. Decreased acetate levels are also observed in patients suffering from multiple sclerosis [25].

We report 35 % decrement of glucose in aged rats as compared with the young, which may be due to the liver being unable to release glucose from glycogen in aged rats [26]. The reduction of glucose is strongly linked with insufficient ATP generation *via* glycolysis and TCA cycle in aged rats. Brain requires a disproportionately large amount of energy to sustain the translocation of ions to maintain action potential and for its high biosynthetic activity. It derives most of its energy from glucose. Therefore, reduced glucose and insufficient energy may be responsible for the various behavioral changes in the aged rats

We also found reduced pyruvate levels (12%) in the serum of aged rats as compared with the young rats, which may be due to reduced glucose level or due to the impairment of glycolysis. Pyruvate, together with ATP, is the end product of glycolysis, following which it enters the Kreb's cycle under aerobic conditions. On the other hand, defects in pyruvate metabolism or in the respiratory chain may lead to lactic acidosis, and we observed elevated lactate levels (23%) in the aged rats. The lactate concentration in serum can be used as an indicator of physical condition [27], moreover, as it is the end product of anaerobic glycolysis [28, 29], therefore it can also be related to the impaired muscle coordination in aged rats [30].

We observed elevated creatine level (46.6%) in aged rats as compared with the young rats. There have been reports that increased levels of creatine are also seen in myotonic dystrophy [31]. Creatine and creatinine phosphate are present in numerous tissues and blood and phosphocreatine act as a reservoir for the generation of ATP. Creatine synthesis takes places in liver and kidney and any perturbation in its level reflects altered liver functioning. Therefore, our results demonstrating elevated creatine levels in serum of aged rats may be due to altered energy scenario associated with aging.

^{1}H NMR spectroscopy was also used to detect age related changes in urine. We found elevated hippurate (HA) level (140%) in aged rats and this may be suggestive of a variety of pathological conditions, including up regulation of ammoniagenesis [32], and inhibition of both plasma protein binding [33] and organic anion secretion by the kidney [34]. HA also inhibits glucose utilization in muscles and it may be involved in development of muscular weakness [35, 36]. Furthermore, it can also be correlated positively with neurophysiological indices [37], which suggest that HA induces neurological symptoms, perhaps *via* inhibition of organic anion transport at the blood-brain barrier or blood-cerebrospinal fluid barrier [38].

Creatinine is a known marker of renal functioning and its concentration in the urine increases as renal function decreases. Creatinine excretion increased up to 170 % in aged rats as compared with the young. The elevated creatinine levels observed in urine of aged rats may be the reflection of reduced glomerular filtration capacity with advancing age [39].

Allantoin is a product of oxidation of uric acid by purine catabolism. It is the predominant means by which nitrogenous waste is excreted in the urine of most of the animals. In humans and higher apes, the metabolic pathway for conversion of uric acid to allantoin has been lost, so the former is excreted. We observed significantly higher levels of allantoin in the aged rat urine as compared with young. The elevated allantoin content is again a reflection of increased oxidative stress in the aged rats [40-42].

Marked changes in the neurobehavioral status of aged rats in terms of memory and motor dysfunction were also observed. Altered neurobehavioral function as observed in the present study is correlated with the perturbed cellular and molecular metabolism [43]. Forster and associates also found such behavioral deficit in old mice which correlates to the protein oxidation and impaired energy metabolism [44]. Therefore, on the basis available evidences, it may be stated that an impaired TCA cycle and electron transport chain functioning in aged rats may lead to decreased ATP production, resulting in changes in motor functioning, perturbed memory status and other neurobehavioral changes [45].

CONCLUSION

On the basis of our results of ^{1}H NMR spectroscopic study, it may be concluded that the altered metabolic profiles of the rat serum and urine reflect the perturbed cellular metabolic pathways in the aged rats. These metabolic changes may be interpreted, as the reflection of aging which may be affecting the brain cells leading to cognitive impairment and age associated diseases.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council of Medical Research (ICMR) and Department of Science and Technology (DST), New Delhi, for the financial support.

REFERENCE

[1] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999; 56: 303-08.

[2] Collier TJ, Coleman PD. Divergence of biological and chronological aging: Evidence from rodent studies. Neurobiol Aging 1991; 12: 685-93.

[3] Rapp PR, Amaral DG. Evidence for task-dependent memory dysfunction in the aged monkey. J Neurosci 1989; 9: 3568-76.

[4] Rapp PR. Recognition memory deficits in a subpopulation of aged monkeys resemble the effects of medial temporal lobe damage. Neurobiol Aging 1991; 12: 481-86.

[5] Hollyday R. Understanding aging. Cambridge University Press: 1995.

[6] Williams RE, Lenz EM, Rantalainen M, Wilson ID. The comparative metabonomics of age-related changes in the urinary composition of male Wistar-derived and Zucker (fa/fa) obese rats. Mol Biosyst 2006; 2:193-202

[7] Conti F, Manganaro M, Miccheli A. Metabolomics and medical practice. Clin Ter 2006; 157: 549-52.

[8] Hodson MP, Dear GJ, Roberts AD, *et al*. A gender-specific discriminator in Sprague-Dawley rat urine: the deployment of a metabolic profiling strategy for biomarker discovery and identification. Anal Biochem 2007; 362:182-92.

[9] Jiang ML, Han TZ, Pang W, Li L. Gender and age-specific impairment of rat performance in the Morris water maze following prenatal exposure to an MRI magnetic field. Brain Res 2004; 995:140-44.

[10] Wienkers LC, Steenwyk RC, Mizsak SA, Pearson PG. *In vitro* metabolism of tirilazad mesylate in male and female rats. Contribution of cytochrome P4502C11 and delta 4-5 alpha-reductase. Drug Metab Dispos 1995; 23 (3): 383-92

[11] Lindon JC, Nicholson JK, Holmes E, Everett JR. Metabonomic: metabolic processes studied by NMR spectroscopy of biofluids. Concepts in Magnetic Resonance 2000; 12: 289-320.

[12] Waters NJ, Holmes E, Willianms A, Waterfield CJ, Farrant RD, Nicholson JK. NMR and patterns recognition studies on the time related metabolic effects of α-nephthyliisothiocyanate on liver, urine and plasma in the rat: an integrative metabonomic approach. Chem Res Toxicol 2001; 14: 1401-12.

[13] Solanky KS, Bailey NJC, Beckwith-Hall BMB, *et al.* Application of biofluid proton nuclear magnetic resonance based metabonomic techniques for the analysis of biochemical effects of dietary isoflavones on human plasma profile. Anal Biochem 2003; 323: 197-204.

[14] Holmes E, Nicholis AW, Lindon JC, *et al.* Development of a model for classification of toxin induced lesions using proton NMR spectroscopy of urine combined with pattern recognition. NMR Biomed 1998; 11: 235-44.

[15] Gupta A, Dwivedi M, Gowda GAN, *et al.* Rapid diagnosis of *Pseudomonas aeruginosa* induced urinary tract infection: use of Proton NMR spectroscopy. NMR Biomed 2005; 18: 293-99.

[16] Gupta A, Dwivedi M, Gowda GAN, *et al.* ^{1}H NMR spectroscopy in the diagnosis of Klebsiella pneumoniae-induced urinary tract infection. NMR Biomed 2006; 19: 1055-61.

[17] Nicholson JK, Wilson ID. High-resolution proton magnetic resonance spectroscopic of biological fluids. Prog Nuclear Mag Reson Spectrosc 1989; 21: 492-501.

[18] Bala L, Gowda GAN, Ghoshal UC, Misra A, Bhandari M, Khetrapal CL. ^{1}H NMR spectroscopic method for diagnosis of malabsorption syndrome: a pilot study. NMR Biomed 2004; 17: 69-75.

[19] Tripathi S, Somashekar BS, Mahdi AA, *et al.* Aluminum mediated metabolic changes in rat serum and urine: A proton magnetic resonance study. J Biochem Mol Toxicol 2008; 22: 119-27.

[20] Lindon JC, Nicholson JK, Everett JR. NMR spectroscopy of biofluids. Annual reports on NMR, Academic Press: London 1999; 38: 1-88.

[21] Nicholls DG. Mitochondrial bioenergetics, aging, and aging-related disease. Int J Biochem Cell Biol. 2002; 34:1372-81.

[22] Hagopian K, Ramsey JJ, Weindruch R. Krebs cycle enzymes from livers of old mice are differentially regulated by caloric restriction Exp Gerontol 2004; 39: 1145-54.

[23] Spindler SR. Calorie restriction enhances the expression of key metabolic enzymes associated with protein renewal during aging. Ann N Y Acad Sci 2001; 928: 296-304.

[24] Wu H, Zhang X, Li X, Li Z, Wu Y, Pei F. Studies on the acute biochemical effects of $La(NO_3)$ using ^{1}H NMR spectroscopy of urine combined with pattern recognition. J Inorg Biochem 2005; 99: 644-50.

[25] Davies SE, Newcombe J, Williams SR, McDonald WI, Clark JB. High resolution proton NMR spectroscopy of multiple sclerosis lesions. J Neurochem 1995; 64: 742-48.
[26] Vangala S, Tonelli A. Biomarkers, metabonomics, and drug development: can inborn errors of metabolism help in understanding drug toxicity? AAPS J 2007; 20; 9 (3):E284-97
[27] Siciliano PD, Lawrence LM, Danielsen K, Powell DM, Thompson KN. Effect of conditioning and exercise type on serum creatine kinase and aspartate aminotransferase activity. Equine Vet J 1995; 18: 243-47.
[28] Kronfeld DS, Ferrante PL, Taylor LE, Custalow E. Blood hydrogen ion lactate concentrations during strenuous exercise in the horse. Equine Vet J 1995; 18: 266-69.
[29] Lawrence LM, Hintz HF, Soderholm LV, Williams J, Roberts AM. Effect of time of feeding on metabolic response to exercise. Equine Vet J 1995; 18: 392-95.
[30] Evans DL, Rainger JE, Hodgson DR, Eaton MD, Rose RJ. The effects of intensity and duration of training on blood lactate concentrations during and after exercise. Equine Vet J 1995;18: 422-25.
[31] Chang L, Ernst T, Osborn D, Seltzer W, Leonido-Yee M, Poland RE. Proton spectroscopy in myotonic dystrophy correlations with CTG repeats. Arch Neurol 1998; 55: 305-11.
[32] Dzurik R, Spustova V, Krivosikova Z, Gazdikova K. Hippurate participates in the correction of metabolic acidosis. Kidney Int Suppl 2001; 78: 278-81.
[33] Sakai T, Takadate A, Otagiri M. Characterization of binding site of uremic toxins on human serum albumin. Biol Pharm Bull 1995; 18:1755-61.
[34] Boumendil-Podevin EF, Podevin RA, Richet G. Uricosuric agents in uremic sera. Identification of indoxyl sulfata and hippuric acid. J Clin Invest 1975; 55: 1142-52.
[35] Spustova V, Cernay P, Golier I. Inhibition of glucose utilization in uremia by hippurate: liquid chromatographic isolation and mass spectrometric and nuclear magnetic resonance spectroscopic identification. J Chromatogr 1989; 490:186-92.
[36] Spustova V, Dzurik R, Gerykova M. Hippurate participation in the inhibition of glucose utilization in renal failure. Czech Med 1987; 10: 79-89.
[37] Schoots AC, De Vries PM, Thiemann R, Hazejager WA, Visser SL, Oe PL. Biochemical and neurophysiological parameters in hemodialyzed patients with chronic renal failure. Clin Chim Acta 1989; 185:91-107.
[38] Ohtsuki S, Asaba H, Takanaga H, Deguchi T, Hosoya K, Otagiri M, Terasaki T. Role of blood-brain barrier organic anion transporter 3 (OAT3) in the efflux of indoxyl sulfate, a uremic toxin: its involvement in neurotransmitter metabolite clearance from the brain. J Neurochem 2002; 83:57-66.
[39] Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem 1992; 38: 1933-53.
[40] Zitnanová I, Korytár P, Aruoma OI, *et al*. Uric acid and allantoin levels in Down syndrome: antioxidant and oxidative stress mechanisms? Clin Chim Acta 2004; 341:139-46.
[41] Yardim-Akaydin S, Sepici A, Ozkan Y, Torun M, Simşek B, Sepici V. Oxidation of uric acid in rheumatoid arthritis: is allantoin a marker of oxidative stress? Free Radic Res 2004; 38: 623-8.
[42] Mikami T, Kita K, Tomita S, Qu GJ, Tasaki Y, Ito A. Is allantoin in serum and urine a useful indicator of exercise-induced oxidative stress in humans? Free Radic Res 2000; 32: 235-44.
[43] Huang C, Mattis P, Perrine K, Brown N, Dhawan V, Eidelberg D. Metabolic abnormalities associated with mild cognitive impairment in Parkinson disease. Neurology 2008; 70:1470-77.
[44] Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, Sohal RS. Age dependent losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc Natl Acad Sci USA 1996; 93: 4765-69.
[45] Palaniappan AR, Dai A. Mitochondrial ageing and the beneficial role of alpha-lipoic acid. Neurochem Res 2007; 32:1552-58.

Received: April 24, 2008 Revised: May 25, 2008 Accepted: June 10, 2008

© Mahdi *et al*.; Licensee *Bentham Open*.

This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.5/), which permits unrestrictive use, distribution, and reproduction in any medium, provided the original work is properly cited.