Original Paper

Effect of Alcohol on Carbohydrate Metabolic Profiles in Rat Kidney: A Study with Reference to Aging

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ABSTRACT

This study was conducted to know the influence of alcohol and aging on carbohydrate metabolic profiles in the kidney tissue of control and experimental male albino rats.

Alcohol significantly decreased the levels of glucose, total proteins, total carbohydrates and increased free amino acids, pyruvate and lactate in the kidney tissue of both age groups of rats.

Aging induced the elevation of the levels of glucose, total carbohydrates, free amino acids and lactate, while it decreased the total protein and pyruvate contents. This suggests rapid utilisation of carbohydrates and degradation of proteins to meet energy demands under alcoholic stress. Thus more alterations were observed in the carbohydrate metabolic profiles in kidney of both age groups of experimental rats.

Key Words: Alcohol; Aging; Kidney; Carbohydrate metabolism

Introduction

Alcoholism is a serious problem for any age group that can induce pathological effects on several important systems of the body, especially the central nervous, cardiovascular, and hepato-renal systems.[1] It is well established that heavy, long-term alcohol consumption plays a major role in the development of irreversible liver damage. Alcohol also directly affects the kidneys by altering their form and function.[2] Excessive consumption has been found to reduce the amount of potassium excreted by the kidney.[3] Alcohol and its metabolite (acetaldehyde) also directly affect magnesium exchange in the kidney tubules. Protein modification in kidney is also induced by alcohol.[4]

Aging is associated with changes in physical characteristics and the decline of many physiological functions.[5] Normal senescence is associated with a number of changes in the composition and functioning of the human body.[6] Aging is associated with spontaneous degenerative changes of renal function and structure. Renal aging in humans and rodents is associated with a spontaneous and progressive decline of kidney function and structural changes in medulla and cortex.[7] Aging can cause histological, functional and molecular changes in the kidney.[8] During alcohol toxicity, stress is induced by the free radicals, and senescence may cause the a fall in energy requirements. Carbohydrate metabolic profiles are important for the organism to recover from alcohol toxicity by producing energy.

In view of the importance of carbohydrate metabolic profile during alcohol toxicity, it was felt desirable to study the influence of long-term consumption of alcohol on carbohydrate metabolic profiles in the kidney of two different age groups of male albino rats.

Materials and Methods

Animal Care and Treatment: Pathogen free, Wistar strain male albino rats (n = 24) of two different age groups, i.e., young (3 months old) weighing 170±10gm, and moderately aged/old (18 months old) weighing 240±10gm

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were used in the current investigation. The rats were housed in clean polypropylene cages, 6 rats per cage, and maintained in a temperature controlled room (27± 2^0C) with a photoperiod of 12 hrs light and 12 hrs dark cycle. The rats were fed with a standard rat pellet diet, and water *ad libitum*.

Chemicals: All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fischer (Pittsburgh, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), and Qualigens (Mumbai, India).

Grouping of Animals: Age-matched rats were divided into 2 groups of six rats in each group and treated as follows:
Group I: Normal Control, (NC) - This group of rats received normal (0.9%) saline orally via orogastric tube for a period of 2 months.

Group II: Alcohol Treated (AT) -Six rats received 20% alcohol at a dose of 2.0 gm/kg bodyweight via orogastric tube for a period of 2 months.

After completion of two months of treatment, and after 24 hrs of the last treatment, the animals were sacrificed by cervical dislocation, and kidney tissue was excised at 4^0C. The tissues were washed with ice-cold saline, and immediately stored in deep freezer at -80^0C for further biochemical analysis. The selected carbohydrate metabolic profiles such as total carbohydrates, total proteins, glucose, and free amino acids were estimated by the methods of Carroll et al 1956,[9] Lowry et al 1951,[10] Mendal et al 1954[11] and Moore & Stein 1954[12] respectively.

Statistical Analysis: The data were expressed as mean values with their SD. Readings of the four different groups were compared using two-factor design ANOVA with replication. Statistical analysis was performed using MS Office, and Excel Software, and the data were analyzed for the significance of the main effects (factors), and treatment along with their interactions. Differences were considered significant at $p< 0.001$.

Results

The levels of total carbohydrates, total proteins, and glucose were decreased, while free amino acids, pyruvate and lactate were increased in alcohol-treated rats of both age groups. Total carbohydrates, free amino acids, and glucose levels were increased in older rats, whereas total proteins levels were decreased (**Table1**).

The results show that alcohol alters the carbohydrate metabolic profiles in the kidney tissue.

Discussion

In the present study, it was observed that the kidney total protein content was decreased with aging process, and in alcohol-treated rats of both age groups. Pacy et al[13] and Preedy & Peters[14] reported decreased protein synthesis and translational efficiency in alcohol-treated rats as compared to control values. Vary et al[15] reported that the rate of protein synthesis is diminished after chronic alcohol consumption through changes in mRNA translation, initiation and elongation.

In this study, very low levels of total protein content was observed in the kidneys of both age groups of rats, which may be due to decreased protein synthesis, or changes that occur in translation and transcription process due to aging. Lewis et al[16] reported a progressive decrease in the rate of whole body protein synthesis during aging. In the present investigation, it was observed that aging results in slight decrement in total proteins in kidney, which may be due to decreased synthesis of proteins in the older animals.[17] The alterations in the involvement of amino acids in protein synthesis contribute to the reduction of protein metabolism in aging tissues. Age-related slowing down and impairment in total proteins appear to play a role in the expression of cellular senescence.[18-19] Forster et al[20] found an age-related increase in protein oxidative damage measured as increased carbonyl content and decreased sulfhydryl content in homogenates of brain, heart and kidney. Goodman et al[21] suggested that aging is accompanied by a decrease in the turnover of whole body protein. The decrease in total protein content may be due to the decreased mitochondrial protein synthesis rates, which indicate a decline in endurance capacity of kidney.[22] Goldspink & Kelly[23] reported decreased protein turnover in liver and kidneys of rats during senility.

The significant decrease in total carbohydrate levels in the kidneys of both young and old rats after alcohol treatment suggests possible utilization of carbohydrates to meet energy demands during alcohol toxicity. This is possible due to the products of alcohol metabolism that in-

Table 1 Changes in Carbohydrate, Glucose, Protein, Free Amino Acids, Lactate & Pyruvate Concentrations in the Kidney of Normal and Alcohol-treated Rats of Two Age Groups

Parameter	Normal Control (NC)		Alcohol-treated (At)	
	Young (3M)	Old (18M)	Young (3M)	Old (18M)
Total Carbohydrates[a]	51.56 ± 2.15	52.44 ± 0.32 (+1.70)	34.36 ± 1.24* (-33.35)	35.24 ± 1.55* (-32.79)
Glucose[b]	1.19 ± 0.01	1.22 ± 0.006 (+1.96)	0.72 ± 0.03* (-39.52)	0.91 ± 0.05* (-24.76)
Total Proteins[c]	526.22 ± 3.47	474.19 ± 2.54 (-9.88)	439.04 ± 2.25* (-16.56)	436.10 ± 1.82* (-17.144)
Free amino Acids[d]	3.39 ± 0.102	3.56 ± 0.03 (+5.01)	3.57 ± 0.058* (+5.35)	3.73 ± 0.04* (+4.91)
Lactate[e]	1.95 ± 0.036	2.15 ± 0.03 (+10.25)	2.26 ± 0.16* (+15.41)	2.40 ± 0.12* (+59.23)
Pyruvate[f]	193.85 ± 1.098	149.97 ± 1.81 (+22.63)	196.14 ± 2.17* (+1.17)	152.88 ± 1.72* (+1.94)

[a] The values are expressed as mg of glucose/gram wet weight of the tissue; [b] The values are expressed as mg of glucose/gram wet weight of the tissue; [c] The values are expressed in mg of protein /gram wet weight of the tissue; [d] The values are expressed in mg of FAA/gram wet weight of the tissue; [e] The values are expressed in mg of lactate/gram wet weight of the tissue; [f] The values are expressed in μmoles of pyruvate formed/gm wet wt of the tissue; Values in the parentheses denote percent change over normal control; * F values are significant at P<0.01.

hibit the formation of glucose from other compounds, such as amino acids.[24] These findings support the present results that the utilization of carbohydrates is more in older rats than the younger ones. It was also observed that aging results in slight elevation in total carbohydrates in the kidney which may be due to decreased metabolic utilization in the older animals. The impaired alterations in the activities of enzymes involved in carbohydrate metabolism contribute to the reduction of carbohydrate catabolism, and elevation in age-related accumulation of tissue carbohydrates. Slowing down and impairment in carbohydrate metabolism appear to play a role in the expression of cellular senescence during aging process.[25]

In the present study, a significant elevation in the glucose levels observed in the kidney of older rats when compared to their respective control of both age groups of rats may be due to low rate of glucose utilization. In the younger rats, the glycolytic pathway is at a higher rate, leading to decreased glucose levels in the tissues to meet the energy demands. According to Sticker et al[26] and Fallon,[27] the operator of glucose catabolic pathway and associated systems decrease during aging, and this could be the reason for high levels of glucose observed in older rats. This study also revealed that a depletion in the glucose levels occurs with alcohol treatment. Lietz et al[28] reported that amino acids are important measures of glucose in the kidney. Renal cortical tubules produce glucose from amino acids efficiently only in the presence of glycerol or lactate, and either fatty acids or ketone bodies.[28] Alcohol-induced decline of glucose formation has also been observed in healthy humans[29] resulting in a risk of alcoholic hypoglycaemia.[30-31] Gluconeogenesis in liver is decreased by alcohol mainly due to elevation of NADH: NAD^+ ratio.[31-32]

The findings in the present study are in agreement with Siler et al.[29] Lieber[32] reported that inhibition of glucose formation in the liver is thought to be responsible for hypoglycaemic alcohol action. Not only the liver, but the kidney also is an important organ to produce glucose in view of the whole body glucose metabolism.[33] The significant decrease in the glucose levels in kidneys of rats after alcohol treatment suggests possible utilization of glucose from shock to meet energy demands during alcohol toxicity. The significant decrease in the glucose levels in kidneys of both age groups of rats after alcohol treatment suggests possible utilization of glucose to meet energy demands during alcohol toxicity.

Levels of kidney pyruvate and lactate were increased in alcohol-treated rats in both the age groups. Lactate levels were higher in the kidney of older rats as compared to younger rats, whereas pyruvate was much lower. This indicates that the slight decrease in pyruvate levels in kidney tissue with advancement of age may be due to greater rate of its formation or slow oxidation of the pyruvate to lactic acid.[34] Elevated levels of pyruvate and lactate were found in the kidney of alcohol-treated rats of both age groups, which suggests that glycolytic pathways are resorted to, due to unregulated activities of the enzymes involved in glycolysis during alcohol treatment.

Increased amounts of free amino acids were found in kidneys of alcohol-treated group of rats in both age groups. The total free amino acid levels increased with age when compared to younger rats. Obled & Arnal[35] suggest that, with advancement of age, protein synthesis decreases and FAA concentration is increased. The elevation in the FAA levels in the older rats may be due to the decreased uptake of amino acids into proteins. Enhanced catabolism during aging might be responsible for elevated amino acid levels in kidney tissues in aged animals.

In the final analysis, it can be concluded that age elevates free amino acids and lowers protein synthesis in rats, by including tissue proteolysis. This indicates that the uptake of amino acids gets reduced during protein synthesis, and hence more amounts of FAA are found in alcohol treated rats of both age groups. Vary et al[15] have reported that protein synthesis is diminished after chronic alcohol consumption through changes in mRNA translation, initiation and elongation.

From the above, it can be concluded that carbohydrate metabolic profiles in the present study were altered during aging process. However, with alcohol consumption these metabolic profiles were much more altered in older rats than in younger rats. This is due to increased energy demands during alcohol toxicity. Hence, consumption of alcohol during old age is much more deleterious.

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