IMPACT OF EXERCISE TRAINING ON CARBOHYDRATE METABOLIC PROFILES IN THE KIDNEY TISSUE OF MALE ALBINO RATS

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ABSTRACT

The present study was designed to investigate the role of exercise training on carbohydrate metabolic profiles in the kidney tissue of two different age groups of rats. The age-matched Wistar strain male albino rats were evenly divided into 4 groups, normal control young (NC-Y), normal control old (NC-O), exercise trained (Ex T-Y) and exercise trained (NC-O). Exercise training was given to the rats for 2 months. After completion of 2 months exercise training the kidney tissue was isolated and assayed the carbohydrate metabolic profiles like, total carbohydrates (TC), glucose (GL), free amino acids (FAA) lactate (LA) total proteins (TP, and pyruvate (PY). In old rats a significant elevation of TC, GL, FAA, LA levels and significant depletion of TP, PY levels were observed than that of control rats. In both the age groups, exercise training significantly reversed the above metabolic profiles to normal levels due to proper utilization of carbohydrate metabolic profiles. This study, clearly shows that exercise could alter the age related accumulated carbohydrate metabolic profiles and their metabolic products in the body. Hence, exercise may have beneficial role against the aging process.

Key words: - Exercise, Aging, Kidney, Carbohydrate metabolic profiles

INTRODUCTION

Aging is a progressive, predictable process involves the steady decline of organ functioning and changes in physical characteristics and the decline of many physiological functions (Meydani and Evans, 1993). Normal senescence is associated with a number of changes in the composition and functioning of the human body (Sathyaprabha, 2000). Aging can cause histological, functional and molecular changes in the kidney (David et al., 2005). Mitochondrial dysfunction and accumulation of protein damage have been increased during aging (Thomas Lattmann et al., 2005).

Numerous studies suggest that physical exercise improves overall health and behaviour as well as cognitive functions, especially in later life (Dubbert, 2002). Voluntary exercise (VE) ameliorates some of the morphological and behavioural consequences of aging (Van Praag et al., 2005). The benefits of regular physical exercise include reduced risk of cardiovascular disease, cancer, osteoporosis and diabetes (Lee et al., 1997; McCarter, 2000). Physical exercise induces physiological changes in tissues of the body (Baldwin et al., 2000). Regular exercise leads to increasing of body’s oxygen uptake which causes more alterations in the carbohydrate metabolic profiles which reduces the diabetic oxidative damage (Mcc Carter, 2000; Lee et al., 2001). There is lot of literature regarding to the exercise training on carbohydrate metabolic profiles, but there was little information available with regard to role of exercise training with reference to aging. Hence, in the present study an attempt was made to know the role of exercise training on carbohydrate metabolic profiles in young and old rats.

MATERIAL AND METHODS

Animal care and maintenance

Wistar strain male albino rats of two different age groups, that is, young (3 months n = 12) weighing 170 ± 10g and old (18 months n = 12) weighing 240± 10 g, were used in the present investigation. * Laboratory of Exercise Biochemistry, Taipei Physical Education College, Taipei City, 11153, Taiwan.
This study was approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CP CSEA/ dt.17.07.2001; resolution number 9/IAEC/SVU/ 2001/dt. 04.03.2002). The rats in the 3-month age group were considered as “younger” and the 18- month age group were considered as “older” or adult rats as per the life span of Wistar strain (Jang et al., 2001). The rats were housed in clean poly-propylene cages, six rats per cage and maintained under hygienic conditions, temperature controlled room (27 ± 2° C) with a photoperiod of 12-h light and 12- h dark cycle. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai, India) and water ad libitum.

**Treatment to the animals**

Age-matched rats were divided into four groups of six in each, and treated as follows.

**Group I** – Normal Control – Young (NC- Y) : Young rats were put on the treadmill belt for 5 minutes for equivalent handling and were treated with normal saline via orogastric tube.

**Group II** – Normal Control – Old (NC- O) : Old rats were put on the treadmill belt for 5 minutes for equivalent handling and were treated with normal saline via orogastric tube.

**Group III** – Exercise training – Young (Ex T- Y) : Young rats were made to run on the treadmill for about 30 minutes at a speed of 23 m/min/5 days in a week for a period of 2 months utilizing an incremental belt speed. The running program was scheduled between 6.00 AM and 8.00 AM.

**Group IV** – Exercise training – Old (Ex T-O) : Old rats were made to run on the treadmill for about 30 minutes at a speed of 23 m/min/5 days in a week for a period of 2 months utilizing an incremental belt speed. Treadmill was custom-built at University Scientific Instrumentation Centre (USIC), Sri Venkateswara University Campus, Tirupati, A.P, India.

**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 (Pittrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

After completion of two months exercise training and after 24 hrs of the last treatment the animals were sacrificed by cervical dislocation and the kidney tissues was excised at 4°C. The tissues were washed with ice-cold saline, and immediately stored at -80°C for further biochemical analysis.

**Biochemical analysis:**

1. **Total carbohydrates** : The total carbohydrate content was estimated by the method of Carroll et al., (1956). The kidney tissue was homogenized in 10% trichloro acitic acid to prepare 1% (W/V) homogenates. The proteins precipitated were removed centrifuging the homogenates for 15 minutes at 3000g. The clear supernatant was taken for the estimation of total carbohydrates. To 0.1 ml of the supernatant, 5 ml of anthrone reagent was added and kept in boiling water bath for 15 minutes. Then the contents were cooled and read at 620 nm against the reagent blank. The total carbohydrate content was expressed as mg of glucose/gm wet weight of the tissue.

2. **Glucose** : Glucose was estimated by the method of Mendal et al., (1954) The tissues were homogenized in 5.0 ml of 90% (W/V) methanol. The suspension was centrifuged and supernatant containing glucose was decanted into a calibrated centrifuge tube. 10 mg of powdered charcoal was added to this methanol solution. The charcoal does not absorb any hexose present, but will remove organic substances which would otherwise interfere with the colour reaction. The methanol was then removed completely under reduced pressure while heating the tube in a water bath. Deproteinizing solution (5% TCA containing 0.1% Silver Sulphate) was added to the residual aqueous solution, still containing the charcoal to bring the total volume to 5ml. The tube was placed in a boiling water bath for 15 minutes and cooled. The suspension was centrifuged and 1 ml of the clear supernatant was taken for glucose estimation. One ml of clear supernatant was added to 3 ml of concentrated sulphuric acid (AR) in a wide-mouthed test tube and mixed by vigorous shaking. The mixture was heated in a boiling water bath for exactly 6.5 minutes and subsequently cooled under running tap water. The intensity of the pink colour developed was read against the blank at 520 nm in a spectrophotometer (Hitachi, UV-2000). The glucose content was
expressed as mg glucose/gram wet weight of the tissue.

3. Lactate: Lactate in the kidney was estimated by the method of Barker and Summerson (1941) as modified by Huckabee (1961). The unexercised and treadmill – exercised rat kidney were chilled for two hours at 0°C. After chilling, the tissues were homogenized in 10% Trichloro acetic acid (TCA) to prepare a 10% (W/V) homogenate and centrifuge tube, to this one ml of 20% copper sulphate solution was added and the solution was made up to 10ml with double distilled water. One gram of calcium hydroxide was added to the contents and shaken vigorously until there was uniform dispersal of the contents. The tubes were kept for an hour with intermittent shaking and then centrifuged. One ml of supernatant was transferred to a clean dry test tube and 0.5 ml of 4% copper sulphate was added followed by 6ml of concentrated sulphuric acid (H₂SO₄). The contents were mixed well and kept in a boiling water bath for exactly one minute and the precipitate was quickly dispersed by lateral shaking. The tubes were kept at laboratory temperature for 30 minutes and then they were placed in a boiling water bath for 90 seconds. After cooling the tubes, the colour of the solution was read at 560 nm against the reagent blank in a spectrophotometer. The lactate content was expressed as mg/gm wet weight of the tissue.

4. Pyruvate: Pyruvate content of the kidney was estimated by the method of Friedmann and Hangen (1942), 10% homogenates of kidney (W/V) were prepared in 10% TCA and centrifuged at 1000g for 15 minutes. To 0.5 ml of supernatant, 2 ml of dinitrophenyl hydrazine (DNP) solution was added. The contents were made up to 10 ml with 10% sodium hydroxide solution and the colour developed was read at 545 nm against the reagent blank in a spectrophotometer. Pyruvate content was expressed as mmoles/gm wet weight of the tissue.

5. Free amino acids: The total free amino acids were estimated by the method of Moore and Stein (1954). 5% (W/V) homogenates of the tissues were prepared in 10% (W/V) trichloro acetic acid (TCA) and centrifuged the contents at 2000g for 15 min at 4°C. To 0.5 ml of supernatant, 2.0ml of Ninhydrin reagent was added and the contents were exactly boiled for 6½ minutes in a boiling water bath. The contents were cooled to laboratory temperature. The samples were made up to 10 ml with distilled water and the colour intensity was read at 570 nm in a spectrophotometer against the reagent blank. The total free amino acid content was expressed in mg of free amino acids per gram wet weight of the tissue.

6. Total protein: The total protein content was estimated by the method of Lowry et al., (1951). The tissue homogenates were prepared in 10% trichloroacetic acid (TCA) and the contents were centrifuged at 1000 x g for 15 minutes. The residue was dissolved in a known volume of 1N sodium hydroxide. 4 ml of alkaline copper reagent was added to the known amount of this solutions and the mixture was kept at room temperature for a few minutes. Then 0.4 ml of folin phenol reagent was added to the above content. The colour developed was read in spectrophotometer at 600 nm against the reagent blank. Protein content was calculated from a standard graph prepared with bovine serum albumin. The total protein content was expressed in mg/gm wet weight of the tissue.

Statistical analysis
All results are presented as mean±SD. The intergroup variation was measured by two way analysis of variance (ANOVA) and the level of significance was considered at p<0.005

RESULTS AND DISCUSSION
The renal total carbohydrate (TC) contents are represented in Fig. 1. TC levels were low in young (51.56) rats than that of old (52.44) rats. However, exercise training significantly (P<0.005) decreased the TC values in young (41.16) rats when compared to old (42.43) rats. The low levels of TC in the old age group indicated that old animals might be more susceptible to age-induced oxidative damage.

Kidney glucose (GL) levels were found to be low in young age rats than that of old age rats. The significant decrease of GL due to exercise training was more in old rats (1.04) than that of young (1.03) rats. The low levels of TC in the old age group indicated that old animals might be more susceptible to age-induced oxidative damage.

In the present study, a significant (P<0.005) drop in renal total protein (TP) levels were observed in old rats, when compared to young rats. Interestingly, exercise training significantly elevated the TP content in both age groups compared to their respective controls. (Fig. 3).
Figure 1: Changes in **Total Carbohydrates levels** in the Kidney tissue of Normal Control (NC - Y), (NC – O), Exercised (Ex T - Y), and (Ex T - O) (23m/min, 30min/day, 5days/week for 2 months) rats of two age groups.

Figure 2: Changes in **Glucose levels** in the Kidney tissue of Normal Control (NC - Y), (NC – O), Exercised (Ex T - Y), and (Ex T - O) (23m/min, 30min/day, 5days/week for 2 months) rats of two age groups.

Figure 3: Changes in **Total Proteins levels** in the Kidney tissue of Normal Control (NC - Y), (NC – O), Exercised (Ex T - Y), and (Ex T - O) (23m/min, 30min/day, 5days/week for 2 months) rats of two age groups.
The kidney free amino acids (FAA) contents are represented in Fig. 4. FAA contents are low in young (3.39) than that of old rats (3.56) rats. However, exercise training decreased the FAA values in young (1.84) rats when compared to old (2.10) rats.

Renal lactate (LA) content was found to be elevated in old age rats. The significant decrease of LA due to exercise training was more in young subjects (2.32) than that of old (2.54) subjects. Exercise training depleted the LA contents in both age groups. (Fig. 5).

In this study, a significant (P<0.005) low pyruvate (PY) content is observed in old rats than that of young rats. The significant increase of PY due to exercise training was more in young rats (199.36) than that of old (153.71) rats. Exercise training restored the contents in both age groups. (Fig. 6).

In the current investigation, TC content was increased in old age rats than young age rats. Aging results in the slight elevation in total carbohydrates in the kidney which may be due to decreased metabolic utilization in the old animals. The impaired alterations in the activities of enzymes involved in the carbohydrate metabolism contribute to the reduction of carbohydrate catabolism and elevation in age-related accumulation of tissue carbohydrates. Tollefsbol (1987) reported that there were more alterations in carbohydrates with

Figure 4: Changes in **Free amino acids contents** in the Kidney tissue of Normal Control (NC - Y), (NC – O), Exercised (Ex T - Y), and (Ex T - O) (23m/min, 30min/day, 5days/week for 2 months) rats of two age groups.

Figure 5: Changes in **lactate contents** in the Kidney tissue of Normal Control (NC - Y), (NC – O), Exercised (Ex T - Y), and (Ex T - O) (23m/min, 30min/day, 5days/week for 2 months) rats of two age groups.
advancement of aging. The depleted carbohydrate levels in kidney of both young and old rats after endurance exercise suggest possible utilization of carbohydrates to meet the energy demands during exercise. Similar pattern of changes in carbohydrate levels has been reported in several tissues of rat during exercise (Bilwanath, 1996). Endurance exercise training increases anaerobic glycolysis with production of lactic acid so as to compensate decreased synthesis of energy through cellular oxidations (Nageswarappa, 1991). Our results are in agreement with those of Vijayakumar Reddy (1990) and Nageswarappa (1991) who reported decreased total carbohydrate content in different tissues under exercise training.

In the present study, a significant elevation in the glucose levels observed in the kidney of old rats when compared to their respective control of both age group of rats may be due to low rate of glucose utilization. In the young 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., (1995) and Fallon (1999) the operator of glucose catabolic pathway and associated system decreased during aging, because of this reason the high levels of glucose was observed in old rats. Lietz et al., (1999) reported that amino acids were considered to be important measures of glucose in kidney. Several studies reveals that glucose levels are decreased with exercise training. (Bilwanath, 1996; Sailaja 1997). During endurance exercise training the kidney become more active than before. As its activity increases, kidney metabolism does likewise which leads to greater output of carbon dioxide resulting from increased oxidation of glucose. So this might be a reason for the decreased glucose content in the kidney of rat with reference to exercise training.

In the present investigation, it was observed that aging resulted in the slight decrement in total proteins in kidney, which may be due to decreased synthesis of proteins in the old animals (Fu and Nair, 1998). Lewis et al., (1997) reported a progressive decrease in the rate of whole body protein synthesis during aging. The alterations in the involvement of amino acids into protein synthesis contributed to the reduction of protein metabolism in aging tissues. The age related slowing down and impairment in total proteins appeared to play a role in the expression of cellular senescence (Moulias et al., 1999) In this study, elevated total protein content was observed in the kidney of exercised rats in both age groups. But the TP content was more increased in young rats than that of old rats. Boom and Watson (1985) reported increased protein synthesis during exercise. Several workers reported increased rate of synthesis of soluble proteins during exercise (Booth and Thomson, 1991). Sailaja (1997) and Rueca et al., (1999) reported an increased protein synthesis with exercise training in muscle and it might be due to increased generation of energy by ATPases. This increased TP content in young rats may be due to the low oxidative damage in young age rats than young rats.
In the present investigation, more amount of FAA was found in the kidney of old age group than young age group. Obled and Arnal (1991) suggested that, with advancement of age protein synthesis is decreased and FAA concentration is increased. The elevation in the FAA levels in the old rats may be due to the decreased uptake of amino acids into proteins and enhanced catabolism during aging might be the factors responsible for elevated amino acid levels in kidney tissues in aged animals and this was further corroborated by the observations of Obled and Arnal (1991) in muscle tissue. In general, it could be concluded that the age by including tissue proteolysis, elevate free amino acids with a decline in protein synthesis in rats. In the present investigation, very small amount of free amino acids (FAA) were found in the kidney of exercise trained rats of both young and old age groups. Physical exercise is known to stimulate the incorporation of amino acids into the protein of various tissues (Hirai et al., 1995) and at the same time, it suppresses the rate of protein catabolism. Motoyama et al., (1989) reported that with exercise training FAA (total amino acid (AA), glucogenic AA, branched chain AA and ketogenic AA) level may be decreased in normal rats. Wenger et al., (1981) and Colombani et al., (1999) suggested that physical exercise increased the rate of amino acid utilization by muscle for protein synthesis. Such an important role was played by amino acids in protein synthesis during exercise. But in the present findings, it was observed that the free amino acid levels were less in exercise trained rats than in control which may be attributed to functional adaptation.

From the present study, it was visualized that there was a slight increase in pyruvate levels in the kidney tissue during aging, which might be due to greater rate of its formation or slow oxidation of the same to lactic acid (Bilwanath, 1996). Exercise induced lactic acid metabolism has been extensively studied and it was noticed an increase in muscle (Sailaja, 1997) increase in blood lactate and hydrogen ion concentrations (Bilwanath, 1996). Intensive exercise is known to result in a state of oxygen debt and triggers the anaerobic glycolysis resulting in the accumulation of lactate. However, other factors may also contribute to the elevation of lactic acid in blood during exercise. According to Urhausan et al., (1995) and Stuewe et al., (2000) increased glyceraldehyde dehydrogenase, suggest that the increased glycolysis and there by elevated levels of lactic acid. Anitha and Devi (1996) reported the decreased lactate/pyruvate ratios also indicate a shift in the carbohydrate metabolism from glycolysis to citric acid cycle due to increased oxidative capacity of the muscle with exercise. In the present investigation, increased lactate/pyruvate levels were observed in the kidney of exercised rats of both young and old age were in agreement with the above reports.

In this study, it was demonstrated the role of exercise training in young and old age rats with reference to carbohydrate metabolic profiles. Alterations of carbohydrate metabolic profiles in young and old rats was observed, this might be due to proper utilization of these profiles under exercise training to meet the energy demands. Exercise training significantly reversed the age-induced oxidative damages in the aged kidney. Hence, exercise training was beneficial and protected the body from oxidative damage induced by aging.

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REFERENCES


