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Influence of Electrolytes Supplementation on Cardiac and Renal Functions after Prolonged Exercise in Male Rats

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Abstract: Heavy sweating after prolonged exercise can cause body fluid losses and alteration of the body function. The purpose of this study was to identify whether electrolytes supplementation promoting rehydration following exercise induced dehydration in male rats. Animals assigned into four equal groups. GroupI served as control, GroupII animals practiced exercise through training program on treadmill,GroupIII animals exercised on treadmill then supplemented by Rehydran-n content dissolved in water. GroupIV animals exercised on treadmill and then supplemented by Rehydran-n content and (Magnesium + Calcium) citrate dissolved in water. After 6 weeks animals were slaughtered and the harvested serum was used to estimate creatine phosphokinase (CPK), lactate dehydrogenase (LDH) enzymes, kidney function (urea and creatinine), Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+}, P ions and aldosterone. Also heart and kidney tissues were dissected out for histopathological examination. The results revealed an increase in CPK, LDH, urea and creatinine which may be due to oxidative stress.Rehydran-n or Rehydran-n and (Magnesium +Calcium) citrate treatment ameliorates these effects and these values decreased in groups III and IV. Na^+, Cl^- and Mg^{2+} decreased While K^+, Ca^{2+} and P increased significantly in exercised group, electrolyte disorders happen. In group III fluid balance happen and Na^+ and Cl^- restored while K^+ still increased. Magnesium and calcium ions increased significantly in group IV only. Group II showed cardiac and renal tissue damage. Aldosterone level decreased in group II while it increased in group III and IV. In conclusion the restoration of body fluid losses is necessary for optimal cardiac and renal functions during exercise. Assuming that rehydration strategy after exercise is necessary.

Key words: Exercise · Electrolytes · Cardiac enzymes · Kidney function · Aldosterone

INTRODUCTION

During prolonged exercise in neutral or slightly warm environments the maintenance of high sweat rates may lead to progressive dehydration which will be accompanied by impaired performance. Mild dehydration will impair exercise capacity and prevent the athletes/workers from making the best use of their skills. In locomotor sports, such as running or cycling, it has been demonstrated that racetimes at major championship events are generally poor when ambient temperature and humidity are high and sweating is perfuse eating [1].

Severe dehydration is potentially fatal: exercise in the dehydrated state leads to a rapid elevation in core temperature, heart rate and the onset of heat illness. An adequate fluid intake before, during and after exercise can help to avoid the negative effects of dehydration. Fluid requirements will depend on work rate, the ambient climatic conditions and also on individual physiological and biochemical characteristics of the athlete /worker [2]. All the physiological perturbations that can cause early fatigue during exercise, dehydration is arguably the most important, if only because the consequences are potentially life threatening [3].

There is a large amount of evidence showing that exercise induced dehydration has a negative impact on exercise performance and restoration of fluid balance must be achieved after exercise. It is equally well known that muscle glycogen must be restored after exercise so that the subsequent performance not to be negatively affected [4]. Athletes need more nutrients than less active people. They demand more from their bodies than even average fitness buff and so must compensate with the right nutrients from foods or supplements to keep performance and recovery at its peak [5].

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The more intense the exercise or sport, the greater the body's nutrient needs. Athletes who participate in endurance sports that involve more than one hour of consistent activity have specific needs because of what they demand from their bodies. For examples, athletes lose more electrolytes, such as magnesium, potassium and sodium, through perspiration and must diligently replace them [6].

Sports drinks are ideally placed to fill both these roles. It is clear that drinking during exercise improves performance, provided that the exercise is of a sufficient duration for the drink to be emptied from the stomach and be absorbed in the intestine [7].

All physiologic systems in the human body are influenced by dehydration [8]. Sweat electrolyte losses depend on the total sweat losses and sweat electrolyte concentrations. Sweat sodium concentration varies depending upon genetic predisposition, diet, sweating rate,heat acclimatization state and sweat concentrations of potassium, calcium, magnesium and chloride averages. Neither sex, maturation, nor aging appear to have marked effects on sweat electrolyte concentrations; although dehydration can increase the sweat concentration of sodium and chloride. Sweat glands reabsorb sodium and chloride, but the ability to reabsorb these electrolytes dose not increase proportionally with the sweating rate [9]. Of importance for rehydration purposes after exercise is consumption of both an adequate volume (greater than the sweat volume lose) and quantity of sodium. Without both of these, rehydration will be neither rapid nor complete. There is, whoever, no good evidence for the inclusion of any other electrolytes [10].

The aim of this work was to investigate the influence of electrolytes supplementation on cardiac and renal functions after prolonged exercise in male rats.

MATERIALS AND METHODS

Animals: Forty male albino rats weighting 130-150g were obtained from the National Research Center, Egypt. The rats were acclimatized on a stock diet and tap water that were allowed *ad libitum*.

Chemical Treatment: Rehydran-n (Glucose anhydrous 4gm, tri-sodium citrate anhydrous 0.51gm, sodium chloride 0.7gm and potassium chloride 0.3gm) was obtained from Sedico Pharmaceutical Company, 6 October City-Egypt. Also (Magnisium citrate and Calcium citrate) obtained from Tiba Pharmaceutical Company, Egypt. All contents dissolved in 2 liter water and adjusted for daily supplementation dose of Rehydran-n content and (54mg/Kg Magnesium and Calcium 66.5mg/kg of rat body weight)for 45days.Rate intake of drinks was estimated by conversion of human dose to animal dose [11] and on the base of the rat water consumption as follows:

$$\text{Animals dose/200g. B.W.} = \text{Human dose} \times 18/1000$$

Exercised Protocol: The rats accustomed to the treadmill exercise for 3days before actual experiment.The animals in the prolonged exercise groups were exercised at rate 25 m/min for 5 min x 4 with pause of 2 min.The training program continue for 6 weeks in the Faculty of physical therapy, Cairo University, Giza, Egypt [12].

Experimental Design: Animals were randomly divided into four equal groups. GroupI animals served as control group (non-exercised). Group II animals exposed to prolonged exercises. Group III animals exposed to prolonged exercises then supplemented byRehydran-n content (Glucose anhydrous 4gm, tri-sodium citrate anhydrous 0.51gm, sodium chloride 0.7gm and potassium chloride 0.3gm) dissolved in 2 liter water. GroupIV animals exposed to prolonged exercises and then supplemented byRehydran-n content plus (54mg/Kg Magnesium citrate and Calcium citrate 66.5mg/kg of rat body weight) dissolved in 2 liter water for 6weeks. At the end of experiment blood samples were withdrawn by cardiac puncture after anesthetization of rats using diethyl ether blood was collected in clean dry test tubes and centrifuged at 3000rpm for 10minutes and sera were then separated and kept frozen for subsequent biochemical analyses.

Biochemical Analysis: Serum creatin phosphokinase (CPK) and lactate dehydrogenase enzyme (LDH) were determined according to method of Young [13]. Serum urea and creatinine were determined using spectro photometric analysis [14,15]. Also Sodium and Potassium was estimated [16,17]. Chloride and Magnesium levels were evaluated in serum by colorimetric method [18,19]. Total calcium concentration was assayed in serum using colorimetric method [20]. Inorganic phosphorous concentration was determined using spectrophotometric method [21]. Aldosterone hormone level was evaluated by the solid phase radioimmunoassay (RIA) using ^{125}I [22].

Statistical Analysis: All data were statistically analyzed as a one-way analysis of variance using the general Linear Model, SAS software [23]. Duncan, multiple range tests was used to separate the means when significant differences exist. Statistical significance was set at 0.05% probability.

Histological Study: Heart and kidney specimens of different groups were dissected out and imbedded immediately in 15% formalin for histopathological examination. The tissue samples were fixed, stained and investigated by light microscope [24].

RESULTS

The results of heart enzymes in (Table 1) revealed that in exercised group(I) CPK and LDH enzymes increased significantly ($P \leq 0.05$) by 29.3 and 24.6% compared to control group respectively. Rehydran-n treatment in group III decreased these levels and percentage become(4.3%) for CPK while LDH still increased significantly ($P \leq 0.05$) by 27.6% compared to control. In Exercised group supplemented byRehydran-n content +(Mg and Ca)citrate CPK and LDH levels become near to level of control.

Figure (1) microscopic sections of heart from exercised group(1b) showed leucocytic cells infiltration in cardiac myocytes. Whereas other sections from Rehydran-n treatment group(1c) revealed few focal intermuscular inflammatory cells infiltration. While Rehydran-n+ (Mg+Ca)citrate treatment group (1d) showing no histopathological changeslike those in control group(1a).

Table (2) demonstrates that there was a significant increase ($P \leq 0.05$) in urea and creatinine levels of exercised animals comparing to control. Rehydran-n supplementation in exercised group decrease the levels below the control value, also in exercised group supplemented by Rehydran-n+ (Mg+Ca)citrate there is amelioration to the effect of dehydration and the ratios become (6.9 and -5.9) compared to control. Also table (2) shows that sodium ions decreased significantly ($P \leq 0.05$) after exercised while after supplementation by Rehydran-n and Rehydran-n+ (Mg+Ca) citrate in group III and IV the level of sodium ion restored near to the control value. While potassium ions level increased significantly ($P \leq 0.05$) in exercised group.

The supplementation by Rehydran-n and Rehydran-n+ (Mg+Ca) citrate in group III and IV not affected on the level of potassium and not return the value near to control value.

Kidney sections of rat from control group (2a) revealed no histopathological changes. While in prolonged exercising group (2b) showing hyalinosis of glomerular tufts. Moreover in Rehydran-n group (2c) vacuolations of epithelial lining renal tubules. Rehydran-n+ (Mg+Ca) citrate treatment group (2d) congestion of renal blood vessel was observed.

Table 1: Effect of electrolyte supplementation on CPK and LDH in different animal groups

	Groups			
Parameters	(I)Control	(II)Exercised	(III)Exercised+Rehydran-n	(IV)Exercised+Rehydran-n+ Mg+Ca
CPK (U/ml)	931±22.4[b]	1204±14.4[a]	971±40.5[b]	942±23.6[b]
% Change		29.3	4.3	1.1
LDH(U/ml)	457±16.9[b]	569±6.4[a]	583±14.7[a]	462±17.9[b]
% Change		24.6	27.6	1.0

Data are represented as mean± S.E. Means in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 2: Effect of electrolyte supplementation on kidney function (serum urea, creatinine)sodiumand potassium ions in different animal groups

	Groups			
Parameters	(I)Control	(II)Exercised	(III)Exercised+ Rehydran-n	(IV)Exercised+ Rehydran-n+ Mg+Ca
Urea (mg/dl)	32.0±0.75[b]	44.7±1.5[a]	28.5±0.75[c]	34.2±0.67[b]
% Change		39.7	-10.9	6.9
Creatinine(mg/dl)	0.51±0.02[b]	0.57±0.023[a]	0.45±0.006[c]	0.48±0.008[b]
% Change		11.8	-11.8	-5.9
Na^+ (mmol/L)	140.9±0.8[a]	119±3.0[b]	143.8±2.4[a]	140.2±0.7[a]
% Change		-15	2.1	0.0
K^+(mmol/L)	10.6±0.32[b]	13±0.41[a]	13.1±0.24[a]	13.0±0.12[a]
% Change		22.64	22.64	23.6

Data are represented as mean± S.E.Means in the same row with different superscripts are significantly different ($P \leq 0.05$). Percentage of change was calculated in comparison with control.

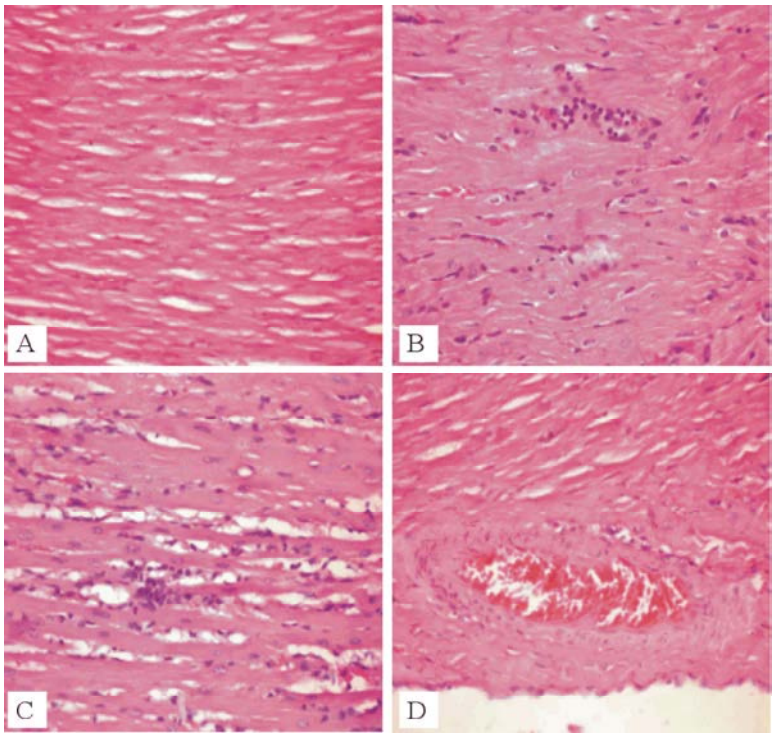

Fig. 1: Sections of heart:(a)Heart tissues of rat from control group showing no histopathological changes.(b)Heart tissues of rat from prolonged exercised group showing few leucocytic cells infiltration in cardiac myocytes. (c) Heart tissues of rat from prolonged exercised+ rehydran group showing few focal intermuscular inflammatory cells infiltration. (d) Heart tissues of rat from prolonged exercised+ rehydran+(Mg+Ca)citrate group showing no histopathological changes (H and E; X=400).

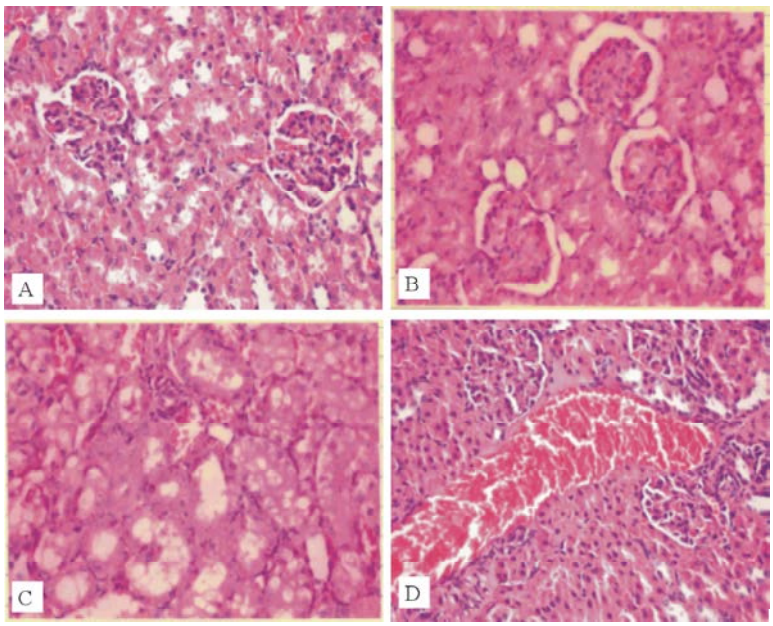

Fig. 2: Sections of kidney rats(a) Kidney of rat from control group showing no histopathological changes. (b)Kidney of rat from exercised+ rehydran+ Mg acetate+Cacl group showing hyalinosis of glomerular tufts. (c)Kidney of rat from exercised+ rehydran group showing vaculations of epithelial lining renal tubules(d)Kidney of rat from exercised group showing congestion of renal blood vessel.(H and E; X=400).

Table 3: Effect of electrolyte supplementation on serum chloride, magnesium, calcium and phosphorous in different animal groups

	Groups			
Parameters	(I)Control	(II)Exercised	(III)Exercised+ Rehydran-n	(IV)Exercised+ Rehydran-n+ Mg+Ca
Cl^- (mmol/L)	125.9±1.2 [b]	99.9±2.4 [c]	132.9±2.4 [a]	125.3±1.2 [b]
% Change		-20.7	5.6	0.4
Mg^{2+} (mmol/L)	5.91±1.17 [b]	4.81±0.16 [c]	6.2±0.14 [b]	7.0±0.27 [a]
% Change		-18.6	4.91	18.4
Ca^{2+} (mg/dl)	4.75±0.18 [c]	8.1±0.27 [b]	5.1±0.1 [c]	8.9±0.2 [a]
% Change		72.3	8.51	89.1
P(mg/dl)	10.6±0.54 [c]	13.4±0.13 [b]	14.2±0.22 [a b]	14.8±0.51 [a]
% Change		26.4	34.0	39.6

Data are represented as mean± S.E. Means in the same row with different superscripts are significantly different (P≤0.05). Percentage of change was calculated in comparison with control.

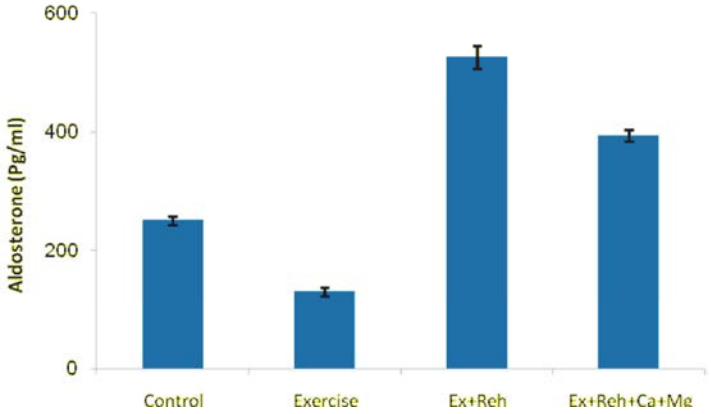

Fig. 3: Effect of electrolyte supplementation on aldosterone hormone level in different animal groups.

Data in table (3) showed a significant decrease (P≤0.05) in chloride ions in group II exposed to prolonged exercise, the percentage was currently lower by 20.7% compared to control. While treatment by Rehydran-n and Rehydran-n+ (Mg+Ca) citrate enhance the levels of chloride in both groups by (5.6 and 0.4%). Moreover Mg ions decreased significantly (P≤0.05) in exercised group by -18.6%. While Rehydran-n and Rehydran-n+ (Mg+Ca) citrate treatment increased the level by (4.91 and 18.4%) in both groups respectively compared to control. Also calcium and phosphorous levels in table (3) show variable significant increase (P≤0.05) by (72.3 and 26.4%) in exercised groups compared to control. There were significant increase in Ca by (8.51 and 89.1) in groups treated by Rehydran-n and Rehydran -n+ (Mg+Ca) citrate respectively. While phosphorous increased significantly by 34 and 39.6% comparing to control.

Fig. (3) Shows that there was a significant decrease(P≤0.05) in aldosterone level in exercised group by 47.4%, while treatment by Rehydran-n and Rehydran-n+ (Mg+Ca) citrate increased the level of aldosterone in both groups by (109 and 57%) respectively comparing to control.

DISCUSSION

The present results are in accordance with the exhausted exercised ratsresulted in an increased growth in serum CPK activity. This increase, however as markedly reduced in the rats after administration of antioxidant [25]. For instance, 16h exercise in rats caused a marked rise in activity levels of serum LDH. Increase in serum LDH activity is mainly due to release from heart and skeletal muscles into blood stream [26]. Moreover rotating drum exercise in rats for 16hr resulted in increase in LDH activities [27]. Different types of stressors cause an increase in activities of serum creatine phosphokinase and lactate dehydrogenase in humans and animals which is an indication of tissue damage [28]. The acute exercise resulted in a significant increase in LDH and CPK. The exhaustive exercise induced muscle damage [29].

Exercise induced oxidative stress was also repeated thoroughbred racehorses after 1000 race at maximum velocity [30]. Exhausting or moderate exercise in rats may increase reactive oxygen species (ROS) production exceeding the capacity of antioxidant defenses [31]. Physical exercise may lead to increase in relative weight of heart and myocytes hypertrophy. The heart is not the only organ that presents adaptation alteration after exercising [32]. Daily intensive exercise caused hypertrophy in the left ventricular heart wall and originated collagen deposition in the right ventricle. Additionally long-term intensive exercise induced a significant increase in messenger RNA expression and protein synthesis of the major fibrotic markers in both atria and in the right ventricle [33].

Electrolyte abnormalities are a frequent and potentially hazardous complication in heart failure. This may be due to the pathophysiological alterations seen in the heart failure state leading to neurohumoral activation (stimulation of the renin-angiotensin-aldosterone system. Heart failure may exhibit hyponatremia due to a decrease in water excretion. Along with potassium and calcium, magnesium influences cardiovascular function. Magnesium and potassium deficiencies play an important role in the development of cardiac arrhythmias [6]. Magnesium is essential for the maintenance of intracellular potassium concentration. Although there are conflicting data regarding the prevalence of hypomagnesemia lead to chronic heart failure than in normal controls. As magnesium and potassium are mainly intracellular ions. There was no correlation between the intracellular electrolyte content and the electrolyte levels in plasma, either for mononuclear cells or erythrocytes or for myocardial and skeletal muscle. Low magnesium and potassium concentrations increase cardiac glycoside toxicity. In contrast, elevated levels of magnesium decrease the sensitivity of human myocardium to anti-arrhythmogenic actions of cardiac glycosides, without affecting maximally developed tension. The antiarrhythmic action of magnesium is suspected to be mediated by a reduced sensitivity to electrophysiological changes induced by Ca^{2+}, thus indicating Ca^{2+} antagonistic properties of magnesium. Magnesium deficiency has also been implicated in sudden death. Therefore, when treating congestive heart failure, one must consider how to prevent depletion of electrolytes or how to replete potassium and magnesium in deficiency states [34].

The dehydration in body decreases urinary excretion and allows urea and other protein waste products to accumulate in the blood and may lead to increase in urea and creatinine levels. Dehydration also induced oxidative stress [35]. Sodium reduction causing hyponatermia, which is one of the most common electrolyte disorders. Low sodium level may be triggered by inadequate dietary intake of sodium and important of adrenal gland or kidney function [36]. The available evidence indicates that the only electrolyte that should be added to drinks consumed during exercise is sodium, which is usually added in the form of sodium chloride. Extra salting of food is an effective strongly for athletes living and training hard in hot weather conditions [37]. Water is not the ideal post-exercise restoration of fluid balance.It has been demonstrated that a drink's sodium concentration is more important than its osmotic content for increasing plasma volume after dehydration [10].

The cells release potassium into the blood stream and serum levels rise with exercise, possibly instigating fatigue. Sodium is the major ion in the extracellular fluid but potassium is the major ion the intracellular fluid. Potassium may therefore be important in achieving rehydration by aiding retention of water in the intracellular space [38]. The rate of recovery was slowest when the KCl was consumed. The exercise induces loss of intracellular potassium and increase the extracellular potassium, with the changes in intra or extracellular potassium concentration likely to influence force development. Calcium loss was also reported during strenuous exercised [39]. Also the present data are coincident with results of heavy exercise which increase potassium level [10].

Furthermore, the histopathological analyses showed moderate congestion of the kidneys of trained animals at high intensity and moderate renal congestion. The renal responses to physical exercises are related to their intensity, therefore, exercises performed at low intensities increase the urinary flow and the sodium excretion., Actually the primary function of the kidneys is to regulate the volume and composition of the extra cellular liquid and hence, these alterations that occur during the performance of physical exercises may generate hemodynamic changes and changes in the sodium and water excretion. These findings concerning the kidneys the trained animals showing decrease in the number of glomerulus which caused by the hypoxia conditions imposed by the exercises [34].

Hypochloremia usually occurs as a result of sodium and potassium depletion, which caused muscle lesion or spasm [40]. Hypomagnisum may cause muscle weakness. Magnesium is lost in sweat and implicated in muscle cramp. There can be a decline in plasma concentration in

exercise [10]. Ca levels may be elevated according to excessive use of calcium containing supplements. Hyperphosphotemia cause muscle spasms and cramps [40]. Hypocalciumia may cause muscle cramps.

Several reports have shown that duration and/ or intensity of exercise elicit different effects on minerals metabolism and that inadequate status of the body mineral composition can lead to a diminution of performance and endurance both in sportsmen and rats [41]. The drinking of a carbohydrate-electrolyte increased Na and K ions in blood. Thus in terms of minimizing physiological disturbances. Also *ad libitum* drinking beverage was as effective as drinking water during exercise but was more effective in recovery [42]. After exercise-induced dehydration serum electrolyte Na^+, K^+ and Cl^- concentration fell. While after ingestion of drinks subjects were in greater net fluid balance [43].

The treatment with rehydrate (fructose, glucose polymer, calcium, magnesium, sodium, potassium, amino acids and vitamins) resulted in increase in treadmill time relative to that of the dehydrated state. The results indicate that constituents other than water, simple transportable monosaccharides and sodium are important for maximal exercise performance and effective recovery associated with endurance exercised-induced dehydration [44]. Postexercise hydration should aim to correct any fluid loss accumulated during the practice or event. Ideally completed within 2 hours, rehydration should contain water to restore hydration status, carbohydrates to replenish glycogen stores and electrolytes to speed rehydration [45].

Several studies reported that the chief function of aldosterone is the regulation of electrolyte. It regulates salt balance. It increases sodium reabsorption and secretion of potassium by the kidney tubules. It is amineralocorticoid hormone. The rate of increase plasma aldosterone as potassium rises in serum is not much lower at high sodium intakes. Thus the potassium is strongly regulated at all sodium intakes by aldosterone when the supply of potassium is adequate [46]. The plasma value of aldosterone one day after exercise was significantly lowered in football players. On the other hand the pre-exercise ingestion of sodium citrate resulted in decrease in serum aldosterone. The observed effect of Na+ on serum aldosterone level may be mediated by an acute increase in plasma volume and serum Na+ concentration alteration [47]. The hypohydration leads to alterations in secretion of all fluid and electrolyte hormone. The specific mechanisms of these alterations appear to be related directly to the decrease in plasma volume [48]. Maybe such decrease is due to the high plasmatic indices of aldosterone, hormone which progressively increases, reaching up to six times more than the indices observed in resting bodies, as means of keeping the body liquids and the homeostasia [34].

CONCLUSION

It is recommended for athletes to drink plain water better than drinking nothing, but drinking a properly for maintained carbohydrate electrolyte sports drink will allow for an even better exercise performance.

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