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The role of L-arginine and aerobic exercise in experimental renal ischemia reperfusion injury in male and female rats

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Original Article

Abstract

Introduction: Renal ischemia/reperfusion (I/R) injury due to reactive oxygen species (ROS) formation is the main cause of acute kidney damage. Nitric oxide (NO) biosynthesis and oxidative stress are closely related to the pathogenesis of renal I/R injury. This study was undertaken to determine the effects of L-arginine (L-arg) as NO donor and aerobic exercise (EX) and also the combination of L-arg with EX on renal I/R injury in male and female rats.

Methods: 54 male and female Wistar rats were divided into four groups in each gender as control, L-arginine (L-arg), treadmill exercise (EX), and L-arginine plus exercise (L-arg & EX). After 8 weeks of EX, animals were exposed to 45 min of bilateral kidney ischemia followed by 24 hours of reperfusion. We assessed serum creatinine (Cr), Kidney tissue damage score (KTDS), kidney weight (KW), serum nitrite and malondialdehyde (MDA) levels.

Results: L-arg, EX or combination of L-arg &EX caused a significant decrease in serum level of creatinine (Cr) ($P<0.05$). KTDS decreased significantly in all female treated groups and male L-arg treated group ($P<0.05$) when compared with the control group. However, the kidney weight was decreased significantly in male rats ($P<0.05$) at 24 hr post reperfusion, and such observation was not seen in female. No significant differences were detected in serum nitrite levels between the groups, but combination of L-arg &EX decreased the serum level of malondialdehyde (MDA) in female significantly ($P<0.05$).

Conclusion: Pretreatment with L-arg seems to have protective effects against renal I/R injury. The protective effect of exercise against renal I/R injury seems to be less than L-arg but the reno-protective effect of EX increases when combined with L-arg.

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Introduction:

Acute kidney injury is a serious disturbance in the hospitalized patients with high mortality rates (1) that caused tubular, glomerular, interstitial and vascular damage (2). The most obvious causes of acute renal failure is ischemia reperfusion (I/R) injury due to formation of reactive oxygen species (ROS) (3). ROS lead to lipid peroxidation and

decrease antioxidant defense, impair renal endothelial cells and decrease nitric oxide (NO) production (4). L-arg is a necessary substrate in the biosynthesis of NO, and as antioxidant and anti-inflammatory agent may protect the kidney against acute renal I/R injury (5). Previous studies provide evidence that supplementation of L-arg has been beneficial effects in acute and recovery phase of renal I/R (6-10), but only limited information is available on 8 weeks treatment effects of L-arg on renal I/R injury.

Aerobic EX training also improves kidney vasodilation by NO (11), increases anti-oxidant defenses, and diminishes oxidative stress and inflammation (12). It is documented that EX protect kidney against renal I/R injury (13). Ex tends to increase oxidative stress due to higher oxygen consumption (14). Maxwell et al (15). Reported L-arg treatment enhances aerobic capacity and reduces oxidative stress induced by EX.

Therefore, in regarded to effcet of gender on renal I/R injury, we designed this research for evaluating the effect of 8 weeks treatment with L-arg and EX and combination of L-arg & EX in both male and female animal models against kidney I/R injury.

Methods:

Animals used were 56 adult rats, of both sexes. Rats were maintained at temperature $23^{\circ}c \pm 2^{\circ}c$ with a 12 h light-dark cycle. The study was in advance approved by Isfahan University of Medical Sciences Ethics Committee (Code #: IR.MUI.REC.1394.2.253).

56 wistar rats were divided into two distinct groups of males (128.32 ± 3.44 gr) and females (119 ± 3.03 gr) also each group was divided into four subgroups as follows:

*Control (n=6 male, 6 female) rats were housed for 8 weeks, and then underwent renal I/R injury.

*L-arg (n= 8 male,7 female) rats received L-arg (100 mg/kg ip (16), 3 times per week) for 8 weeks before renal I/R injury.

*EX (n=8 male, 7 female) rats were subjected to treadmill exercise (5 days a week) for 8 weeks before renal I/R injury.

*L-arg &EX (n=6 male, 8 female) rats were subjected to receive L-arg and EX as groups 2 and 3 before renal I/R injury.

EX training was performed on motorized treadmill (Technic Azma NT540, Tabriz, Iran) 5 days/week for 8 weeks (17). Rats were habituated to treadmill for three days. The training program was gradually increased intensity from 15m/min for 15 min of the first day until it reached to 26 m/min for 60 min by the end of second week, and with the same setting and EX protocol was continued by the end of 8th week. At the beginning of each session rats were warmed up for 5 min at the speed of 10 m/min and finally after the exercise rats would cool down for 5 min with the speed 10 m/min. This protocol was designed to correspond to 65% maximum oxygen consumption (17).

To induce renal I/R injury, renal artery and vein were occluded for 45 min followed by 24 h reperfusion. Blood sample was obtained and the kidneys were removed for histological staining by hematoxylin and eosin method. To considerate kidney tissue damage score (KTDS) was evaluated presence of tubular atrophy, hyaline casts, ischemic necrosis, vacuolization and debris. The samples were scored from 1 to 4 based on intensity of damage, 0 = normal kidney; 1= minimal damage 5-25% involvement of the cortex or outer medulla); 2= mild damage (25-50% involvement of the cortex or outer medulla); 3= moderate damage (50-75% involvement of the cortex or outer medulla); 4= severe damage (>75% involvement of the cortex or outer medulla).

The serum levels of creatinine (Cr) using quantitative kits (Pars Azmon, Iran), nitrite (stable NO metabolite) using Griess method, and malondialdehyde (MDA) by a manual method were measured.

Results were expressed as mean±standard error of the mean. One way ANOVA followed by LSD for analysis the quantitative data (Cr, KW, MDA and nitrite) and the Kruskal- Wallis and Mann-Whitney tests for qualitative data (KTDS) were used. $P<0.05$ was considered statistically significant.

Results:

Results obtained from this study showed that: serum creatinine (mg/dl) was significantly decreased in all treated groups compared to control groups ($P<0.05$) for male groups (Control 1.07 ± 0.19, L-arg 0.67 ± 0.02, EX 0.74 ± 0.04, L-arg & EX 0.71 ± 0.05) for female groups (Control 1.56 ± 0.24, L-arg 0.74 ± 0.06, EX 1.08 ± 0.11, L-arg & EX 0.96 ± 0.12).

KTDS showed decreased in all treated groups but it was significantly decreased in male L-arg group (1.37 ± 0.18) compared to control group (2.33 ± 0.33 $P<0.05$), and in all female groups compared control ($P<0.05$) (control 2.66 ± 0.21, L-arg 1.71 ± 0.28, EX 1.71 ± 0.18, L-arg & EX 1.50 ± 0.18).

KW was significantly decreased in male treated groups compared to control ($P<0.05$) (control 0.92 ± 0.08, L-arg 0.80 ± 0.02, EX0.76 ± 0.02, L-arg & EX 0.71 ± 0.06).

No significant changes in serum level of nitrite were detected between all the studies groups (figure 1) while combination of L-arg & EX reduced the serum level of MDA significantly in female (0.93 ± 0.19) compared to control ($2.4\pm.67$ $P<0.05$). Although serum MDA were lowered in L-arg groups, and higher in the EX groups compared to control (Figure 1).

The samples of kidney tissue images are shown in Figure 2.

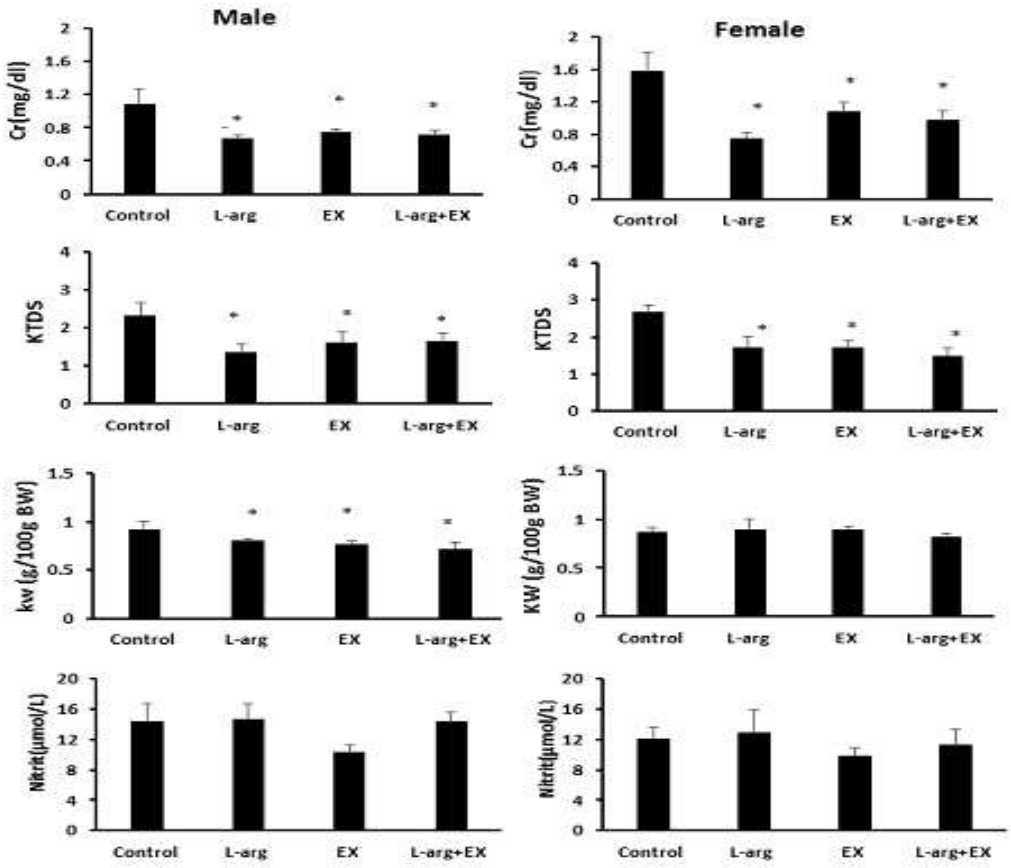

Figure 1. Changes in serum levels of creatinine (Cr) , nitrite and malondialdehyde (MDA), and kidney tissue damage score (KTDS), and kidney weight (KW) g/100g of body weight in male and female rats subjected to renal I/R after 8weeks (45 min ischemia followed by 24 hours of reperfusion) treated with vehicle (control), L-arginine (L-arg 100mg/kg 3days/week ip for 8 week before I/R) , treadmill exercise (EX, treadmill exercise 5 days/week for 8 week before I/R) and both L-arg & EX. (L-arg100mg/kg 3days/week ip along with 5days/week treadmill exercise for 8 weeks beforeI/R)
***:P<0.05 compared to control group**

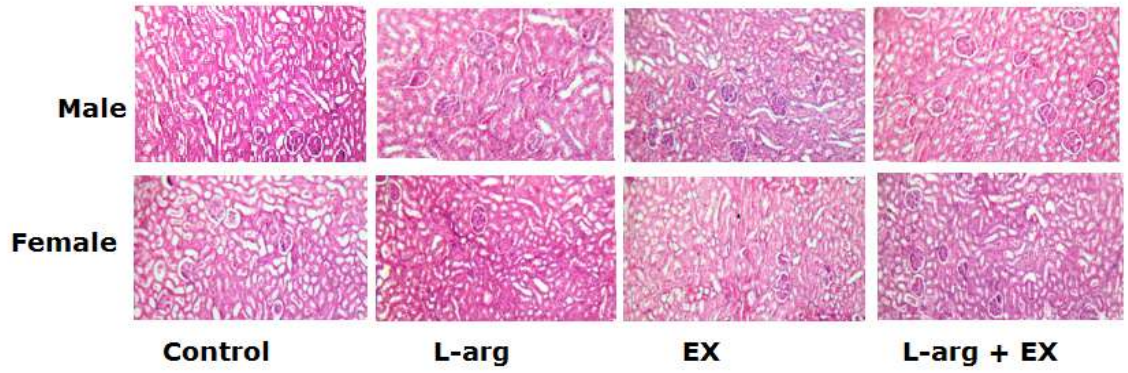

Figure 2. Samples images of kidney tissue in male and female rats subjected to renal I/R after 8weeks (45 min ischemia followed by 24 hours of reperfusion) treated with vehicle (control), L-arginine (L-arg, 100mg/kg 3days/week ip for 8 week before I/R) , treadmill exercise (EX, treadmill exercise 5 days/week for 8 week before I/R) and both L-arg & EX. (L-arg100 mg/kg 3 days/week ip along with 5 days/week treadmill exercise for 8 weeks beforeI/R) Extensive damage was seen in control groups.

Conclusion:

The present study demonstrated that pretreatment with L-arginine supplementation and regular EX protect kidney against I/R injury but the effect of L-arginine alone or combined with exercise in improvement renal I/R injury was more apparent than EX alone.

Previous study showed that the renal I/R injury Characterized by decreases in glomerular filtration rate (GFR), tubular and glomerular damage, impairment in hemodynamic regulation and energy depletion (3). Some other mediators such as cascade of inflammation events involved in pathogenesis of renal damage (18).

Our results are agreement with the other study which suggested that L-arginine supplementation (7) and EX (19) can improve renal dysfunction in I/R. L-arg increase renal plasma flow (RPF) and glomerular filtration rate (GFR) (20). Schneider and et al (8) reported that pretreatment with L-arg improves recovery phase of renal I/R. Also, protective role of EX were reported (21,22). In agreement with our study saad and et al (19). Demonstrated that regular EX before the induction of renal I/R injury significantly improved renal function.

KTDS increase were observed after renal I/R (23). In several studies administration of exogenous L-arg has been shown to protect kidney against ischemic injury (5,8,10,24,25). The same way Moraes and et al (26) reported that EX training improved endothelium-dependent and endothelium-independent kidney vasodilation through NO and Ex induced adaptation in vascular endothelium (27). Ex also increased renal antioxidant capacity (28) and enzymes (29).

It was reported that I/R increased KW that is related to kidney injury and edema due to enhance endothelial permeability (30,31). Our study indicated KW was significantly attenuated only in male treated rats. In female treated rats despite the significant decrease in KTDS and creatinine levels, kidney weight did not decrease. These difference response may be due to sex hormone and estrogen effect on cell proliferation (32). The nitrite level is important to distinguish the function of NO (33). The serum levels of nitrite in the L-arg and L-arg & EX groups were higher than EX group possibly due to increase in eNOS (5). Previous studies reported pre-treatment with L-arg of rats subjected to renal I/R markedly increase tissue NO content (7).

MDA as a marker of lipid peroxidation increased after renal I/R (23). In present study L-arg decreased and EX increased serum MDA in male and female insignificantly. It seems that L-arg had a definite antioxidant effect which showed more powerful when combined with EX. It seems that EX induces more oxidative stress and in EX groups antioxidant system was not adapted to excessive

production of ROS due to I/R. It is supposed that our treadmill EX protocol may not be applied appropriate EX intensity. However, exercise training protocol used in this study had presented cardioprotection and renoprotective (17). The significance of MDA levels in female L-arg & EX group may be related to the antioxidant effect of estrogen hormone (34).

Finally, according to previous research, male are more sensitive to renal I/R injury than female (35), however, no difference in gender was observed in our model when L-arg or EX were administrated against kidney I/R injury.

In our study limitation, we did not measure nitrite and MDA in kidney and serum urea for evaluate change function and structure, it might be powerful to measure the antioxidant enzymes such as superoxide dismutase.

Pretreatment with L-arg alone or in combined with EX were more attenuate renal I/R injury than EX in rats, possibility due to their antioxidant effect without any gender difference.

Conflict of interest:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References:

1. Palant CE, Amdur RL, Chawla LS. Long-term consequences of acute kidney injury in the perioperative setting. Current Opinion in Anesthesiology. 2017;30(1):100-104.
2. Zuk A, Bonventre JV. Acute kidney injury. Annual review of medicine. 2016;67:293-307.
3. Malek M, Nematbakhsh M. Renal ischemia / reperfusion injury; from pathophysiology to treatment. Journal of renal injury prevention. 2015;4(2):20-27.
4. Chatauret N, Badet L, Barrou B, Hauet T. Ischemia-reperfusion: From cell biology to acute kidney injury. Progrès en urologie. 2014;24(Suppl 1):S4-S12.
5. Cherla G, Jaimes EA. Role of L-arginine in the pathogenesis and treatment of renal disease. The Journal of nutrition. 2004;134(10):2801S-2806S.
6. Özülkü M, Aygün F. Effect of L-arginine on Hemodynamic, Biochemical, and Histopathological Outcomes in a New Zealand Rabbit Model of Renal Ischemia–Reperfusion Injury. Journal of Academic Research in Medicine-Jarem. 2016;6(1):24-30.
7. Mohamed AE-HA, Lasheen NN. Comparative study on the protective role of vitamin C and L-arginine in experimental renal ischemia reperfusion in adult rats. International Journal of Physiology, Pathophysiology and Pharmacology. 2014;6(3):153-165.
8. Schneider R, Raff U, Vornberger N, Schmidt M, Freund R, Reber M, et al. L-Arginine counteracts nitric oxide deficiency and improves the recovery phase of ischemic acute renal failure in rats. Kidney International. 2003;64(1):216-225.
9. Salah El Din, Rania A. The protective role of vitamin c and l-arginine on ischemic-reperfusion injury in the renal cortex of adult albino rats: histological and immunohistochemical study. Egyptian Journal of Histology. 2015;38(2):241-252.
10. Chander V, Chopra K. Renal protective effect of molsidomine and L-arginine in ischemia-reperfusion induced injury in rats. Journal of Surgical Research. 2005;128(1):132-139.
11. Pagliaro P, Mancardi D, Penna C. Nitric Oxide Synthase Function in Exercise. Current Enzyme Inhibition. 2008;4(1):37-45.
12. de Moraes R, H Valente R, R Leon I, RO Trugilho M, G Pacheco A, CL Nobrega A, et al. Alterations of the kidney cortex proteome in response to exercise training in normoglycemic and hyperglycemic conditions. Current Topics in Medicinal Chemistry. 2014;14(3):450-461.
13. Moningka NC, Cunningham Jr MW, Sterling M, West CA, Verlander JW, Croker BP, et al. Effects of voluntary wheel running on the kidney at baseline and after ischaemia–reperfusion-induced acute kidney injury: a

strain difference comparison. The Journal of Physiology. 2013;591(5):1313-1324.

14. Yavari A, Javadi M, Mirmiran P, Bahadoran Z. Exercise-induced oxidative stress and dietary antioxidants. Asian Journal of Sports Medicine. 2015;6(1).
15. Maxwell AJ, Ho H-KV, Le CQ, Lin PS, Bernstein D, Cooke JP. L-arginine enhances aerobic exercise capacity in association with augmented nitric oxide production. Journal of Applied Physiology. 2001;90(3):933-938.
16. Gupta V, Gupta A, Saggu S, Divekar HM, Grover K, Kumar R. Anti-stress and adaptogenic activity of L-arginine supplementation. Evidence-based Complementary and Alternative Medicine. 2005;2(1):93-97.
17. Borges JP, França GdO, Cruz MD, Lanza R, Nascimento ARd, Lessa MA. Aerobic exercise training induces superior cardioprotection following myocardial ischemia reperfusion injury than a single aerobic exercise session in rats. Motriz: Revista de Educação Física. 2017;23(SPE).
18. Rovcanin B, Medic B, Kocic G, Cebovic T, Ristic M, Prostran M. Molecular dissection of renal ischemia-reperfusion: oxidative stress and cellular events. Current medicinal chemistry. 2016;23(19):1965-1980.
19. Saad RA. Long term exercise preconditioning protects against renal dysfunction after ischemia reperfusion injury in rat kidneys. Journal of American Science. 2014;10(6):154-161.
20. Klahr S, Morrissey J. L-arginine as a therapeutic tool in kidney disease. Seminars in nephrology. 2004;24(4):389-394.
21. dos Santos Silva KA, da Silva Luiz R, Rampaso RR, de Abreu NP, Moreira ÉD, Mostarda CT, et al. Previous exercise training has a beneficial effect on renal and cardiovascular function in a model of diabetes. PloS one. 2012;7(11):e48826.
22. Leite CF, Rombaldi AJ. Effect of carbohydrate intake and physical exercise on glycogen concentration. Revista Brasileira de Cineantropometria & Desempenho Humano. 2015;17(1):62-72.
23. Malek M, Nematbakhsh M. The preventive effects of diminazene aceturate in renal ischemia/reperfusion injury in male and female rats. Advances in preventive medicine. 2014;2014.
24. Shokeir AA, Barakat N, Hussein AAM, Awadalla A, Abdel-Aziz A, Abo-Elenin H. Role of combination of l-arginine and α-tocopherol in renal transplantation ischaemia/reperfusion injury: a randomized controlled experimental study in a rat model. BJU International. 2011;108(4):612-618.
25. Senbel AM, Omar AG, Abdel-Moneim LM, Mohamed HF, Daabees TT. Evaluation of l-arginine on kidney function and vascular reactivity following ischemic injury in rats: Protective effects and potential interactions. Pharmacological Reports. 2014;66(6):976-983.
26. De Moraes R, Gioseffi G, Nóbrega AC, Tibiriçá E. Effects of exercise training on the vascular reactivity of the whole kidney circulation in rabbits. Journal of Applied Physiology. 2004;97(2):683-688.
27. Padilla J, Simmons GH, Bender SB, Arce-Esquivel AA, Whyte JJ, Laughlin MH. Vascular effects of exercise: endothelial adaptations beyond active muscle beds. Physiology. 2011;26(3):132-145.
28. Moningka NC. Impact of exercise on renal nitric oxide and antioxidant systems: University of Florida; Proquest Dissertations Publishing, 2011.
29. Moningka N, Cunningham M, Sterling M, Baylis C. Impact of 12 wks exercise on renal nitric oxide and antioxidant status: a strain difference comparison. The FASEB Journal. 2010;24(1 Supplement):1059.1.
30. Basile DP, Yoder MC. Renal endothelial dysfunction in acute kidney ischemia reperfusion injury. Cardiovascular & Hematological Disorders Drug Targets. 2014;14(1):3-14.
31. Moeini M, Nematbakhsh M, Fazilati M, Talebi A, Pilehvarian AA, Azarkish F, et al. Protective role of recombinant human erythropoietin in kidney and lung injury following renal bilateral ischemia-reperfusion in rat model. International Journal of Preventive Medicine. 2013;4(6):648-655.
32. Yanes LL, Sartori-Valinotti JC, Reckelhoff JF. Sex steroids and renal disease. Hypertension. 2008;51(4):976-981.

33. Shiva S. Nitrite: a physiological store of nitric oxide and modulator of mitochondrial function. Redox Biology. 2013;1(1):40-44.

34. Bednarek-Tupikowska G. Antioxidant properties of estrogens. Ginekologia Polska. 2002;73(1):61-67.

35. Kang KP, Lee JE, Lee AS, Jung YJ, Kim D, Lee S, et al. Effect of gender differences on the regulation of renal ischemia - reperfusion - induced inflammation in mice. Molecular Medicine Reports. 2014;9(6):2061-2068.

اثر ال آرژنین و تمرین هوازی بر آسیب ایسکمی/ری پرفیوژن کلیوی در رتهای نر و ماده

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مجله پزشکی هرمزگان سال بیست و یکم شماره اول ۹۶ صفحات ۲۷-۲۰

چکیده

مقدمه: *آسیب ایسکمی/ری پرفیوژن کلیوی به دنبال تولید گونه های واکنشی اکسیژن (ROS) از جمله علت آسیب های حاد کلیوی است. بیوسنتز نیتزیک اکساید و استرس اکسیداتیو با پاتوژنز آسیب ایسکمی/ری پر فیوژن مرتبط است. در این مطالعه اثر ال-آرژنین به عنوان پیش ساز نیتزیک اکساید و تمرین هوازی و ترکیب ال آرژینین و ورزش در آسیب ایسکمی/ری پرفیوژن کلیوی در دو جنس نر و ماده مورد بررسی قرار گرفت.*

روش کار: *۵۴ سر رات در دوجنس نر و ماده و در هر جنس در ۴ گروه کنترل، دریافت کننده ال -آرژنین ۸ هفته‌ای، تمرین هوازی ۸ هفته‌ای ترد میل و ترکیب ال -آرژنین و تمرین هوازی ۸ هفته‌ای ترد میل مورد آزمایش قرار گرفت. در پایان ۸ هفته تمام حیوانات تحت ۴۵ دقیقه ایسکمی کلیوی و ۲۴ ساعت ری پرفیوژن قرار گرفتند. سطح سرمی کراتینین، میزان آسیب بافت کلیوی، وزن کلیه،سطح سرمی نیتریت و مالون دی آلدهید مورد بررسی قرار گرفت.*

نتایج: ال -آرژنین، تمرین هوازی ۸ و ترکیب هر دو در مقایسه با گروه کنترل موجب کاهش سطح سرمی کراتینین شد ($P<۰/۰۵$). آسیب بافتی کلیه در تمام گروههای تحت درمان جنس ماده و گروه ال ارژینین جنس نر کاهش معنی‌داری داشت ($P<۰/۰۵$) اما وزن کلیه فقط در جنس نر کاهش داشت ($P<۰/۰۵$). تفاوتی در سطح سرمی نتیریت در گروه‌ها مشاهده نشد اما ترکیب ال -آرژنین و تمرین هوازی ۸ هفته‌ای ترد میل سطح سرمی مالون دی آلدهید را فقط در رات های ماده کاهش داده بود ($P<۰/۰۵$).

نتیجه‌گیری: *به نظر می‌رسد ال آرژنین به تنهایی و یا در ترکیب با ورزش بتوانند عامل موثری برای کاهش آسیب ایسکمی/ری پرفیوژن کلیوی باشند. ورزش به تنهایی اثرات محافظتی کمتری نسبت به ال ارژینین نر برابر آسیب کلیوی نشان داد.*

کلیدواژه‌ها: *ری پرفیوژن، ال آرژنین، تمرین هوازی*

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