32 - EVALUATION OF BIOCHEMICAL PARAMETERS IN FEMALE FISHER RATS SUBJECTED TO SWIMMING TRAINING IN DIFFERENT VOLUMES (30, 60, 120 AND 240 MINUTES).

WANDA MARIA DE FARIA, RINALDO CARDOSO DOS SANTOS,
MARIA LÚCIA PEDROSA, MARCELO EUSTÁQUIO SILVA.
UNIVERSIDADE FEDERAL DE OURO PRETO, OURO PRETO – MG, BRASIL.
mesilva@enut.ufop.br

INTRODUCTION

A study performed by Bauman et al., (2009), states that physical inactivity causes two million deaths annually around the world. Sedentariness, age and heredity are called risk factors for disease onset. Various researches have stressed as much as physical activity has been efficient in the prevention and treatment of chronic diseases such as diabetes, cardiovascular disease and especially obesity, which are caused by a highly caloric diets and sedentary lifestyle (Ciabattari, 2005).

McArdle et al., (1998), verified that the effects of training of moderate volume and intensity exerted positive effects, reducing the risk of development of chronic-degenerative diseases, like cardiopatty and cancer. As a matter of fact even physical activity at relatively low level exerts a positive effect. Studies on Exercise Physiology have utilized animal models aiming at simulating stress conditions observed in humans, seeking a better follow-up of the systemic alterations caused by exercise. Exercise protocols for animals must adequately simulate the situations proposed by the investigations. Amongst the main means used for this purpose are the esteira rolante and swimming. Rats are preferably employed due to easy manipulation and for presenting good response to exercise (Gobatto et al., 2001).

OBJECTIVE

Our objective was to better understand the effects of swimming in an animal model in different volumes (30, 60, 120 and 240 minutes) on biochemical markers of hepatic function, lipid profile and oxidative stress.

MATERIALS AND METHODS

Animals

Forty six 60 day-old female Fisher rats, weighing in average 100g were kept in individual cages, at 25±1ºC under a 12/12 hour light cycle. They were distributed into four groups of nine animals each and subjected to swimming with different volumes (30, 60, 120 and 240 minutes) during five days per week for two months. A sedentary group (10 animals) was kept in the same conditions. During the experiment a commercial ration for laboratory animals, and filtered water were offered ad libitum.

Training

The exercised animals were adapted to the liquid medium (water at 31±1ºC) as follows: 1st and 2nd days, 30 min. in a shallow pool; 3rd and 4th days, two series of 15 x 5 min. interval in a 50 cm deep pool and in the 5th day 30 min. continuously at the same depth of the former day. From the second week to the end of the experiment the time of exercise was increased gradually, starting with 30 min. and at the end of the experiment one group swam in sessions of 30, 60, 120 or 240 min, five days per week. The sedentary animals were subjected to contact with water during 30 min. during the hole experiment, so that they were exposed **to the same handling stress.**

Biochemical evaluation

Biochemical analysis (Total proteins, ALP, AST ALT, creatinine, urea, total cholesterol and fractions, tryglycerides, CK and LDH) were performed using commercial kits (Labtest S.A., Lagoa Santa, MG, Brazil). Free and total sulphydryls were determined according to Sedlak e Lindsay (1968). Paraoxonase was measured according to Beltowski et al., (2002) e Eckerson et al., (1983) and catalase according to Aebi (1984).

Statistical analysis

One Way ANOVA was employed with the post-test of Tuckey using the software Prism® of GraphPad version 4.00 for Windows, GraphPad software, San Diego, California, USA, www.graphpad.com.

RESULTS

Table 1 – Concentration of total proteins, Alkaline phosphatase (ALP) activity, Oxaloacetic (AST) and Piruvic (ALT) transaminases activities, creatine, urea, total, HDL and other fractions (LDL + VLDL)cholesterol, tryglycerides, creatine kinase (CK) activity, lactic deshidrogenase (LDH), paraoxonase ans catalase activities, total and free sulphydryls of sedentary (n=10) and trained (n=9)animals after 14 weeks of experiment.

Parâmetros	Sedentary	T30`	T60`	T120`	T240`
TP (g/dL)	7.29 ± 0.36^{a}	8.19 ± 0.67$^{b, c}$	7.76 ± 0.20$^{a, b}$	8.36 ± 0.20$^{c, d}$	8.78 ± 0.43^{d}
ALP (U/L)	21.76 ± 3.67^{a}	23.05 ± 3.82^{a}	27.44 ± 6.43$^{a, b}$	30.39 ± 6.61^{b}	28.33 ± 5.67$^{a, b}$
AST (U/mL)	22.23 ± 4.45$^{a, b}$	20.32 ± 5.10^{b}	26.13 ± 5.27$^{a, b}$	27.54 ± 5.06^{a}	28.55 ± 5.11^{a}
ALT (U/mL)	8.91 ± 0.92^{a}	10.22 ± 1.86$^{a, b}$	9.89 ± 1.48$^{a, b}$	9.83 ± 1.02$^{a, b}$	11.00 ± 1.32^{b}
Creatinine (µmol/L)	76.89 ± 5.09^{a}	78.18 ± 5.52$^{a, b}$	86.23 ± 3.51^{b}	76.86 ± 3.65$^{a, b}$	80.56 ± 13.06$^{a, b}$
Ureia (mmol/L)	7.07 ± 0.79^{a}	7.22 ± 0.48^{a}	7.67 ± 1.19$^{a, c}$	8.89 ± 0.63^{b}	8.44 ± 0.89$^{b, c}$
TC (mmol/L)	1.89 ± 0.22^{a}	1.52 ± 0.16^{b}	1.67 ± 0.25^{b}	1.36 ± 0.16^{c}	1.33 ± 0.15^{c}
OF (mmol/L)	0.71 ± 0.10^{a}	0.42 ± 0.12^{b}	0.48 ± 0.10^{b}	0.23 ± 0.15^{c}	0.13 ± 0.12^{c}
HDL(mmol/L)	1.18 ± 0.16^{a}	1.10 ± 0.13^{a}	1.19 ± 0.19^{a}	1.13 ± 0.17^{a}	1.20 ± 0.10^{a}
T (mmol/L)	0.57 ± 0.14^{a}	0.56 ± 0.15^{a}	0.57 ± 0.13^{a}	0.55 ± 0.10^{a}	0.57 ± 0.09^{a}
CK (U/L)	1579.28 ±300.95^{a}	1658.13 ± 271.50^{a}	762.73 ± 240.24$^{b, c}$	703.82 ± 265.47^{b}	1099.13 ± 229.09^{c}
LDH (U/L)	334.17 ± 84.00^{a}	153.77 ± 61.54^{b}	307.12 ± 74.59^{a}	229.20 ± 71.55$^{a, b}$	251.14 ± 105.37$^{a, b}$
PON (U/L)	88.88 ± 10.54^{a}	86.00 ± 09.91^{a}	86.82 ± 15.54^{a}	95.00 ± 09.37^{a}	94.00 ± 10.40^{a}
Catalase (U/L)	26.90 ± 25.44^{a}	32.43 ± 14.45^{a}	39.76 ± 25.88^{a}	22.84 ± 13.42^{a}	14.10 ± 23.12^{a}
TS (U/L)	255.09 ± 38.45^{a}	269.16 ± 32.73^{a}	258.58 ± 37.43^{a}	234.50 ± 31.15^{a}	267.01 ± 28.83^{a}
FS (U/L)	60.61 ± 2.23^{a}	58.89 ± 4.69^{a}	61.04 ± 3.68^{a}	53.22 ± 4.43^{b}	50.91 ± 2.47^{b}

Results are expressed as the mean ± standard deviation.*Different letters in the same column ind icate significant difference (P = 0,05). Total Proteins (TP); Total Cholesterol (TC); Other fractions (OF); Tryglycerides (T) ; Total Sulphydryls (TS); Free Sulphydryl (FS).

According to the results presented in table 1 a siginicant increase in the concentration of proteins of the animals of groups T30`, T120` and T240`, in relation to the sedentary ones, was observed. Also, ALP activity was higher in the T120` group as compared to the T30` and the sedentary groups. AST activity was diminished in T30` rats in relation to T120` and T240` but equal to T60` and sedentary. In ALT activity and increase was observed in T240` as compared to sedentary ones but equal to all others. A significant difference was observed in the renal function parameters: creatine concentration was found augmented in T120` in relation to the sedentary group but similar amongst all other groups. Urea was augmented in T120` in relation to the sedentary, T30` and T60` groups. There was a significant decrease in total cholesterol in all trained animals, but amongst T120` and T240` it was more pronounced as compared to the sedentary ones. As for the other cholesterol fractions (VLDL and LDL) it can be observed a significant and gradual decrease according to the increase of physical activity in groups T30`, T60`, T120` and T240` as compared to the sedentary ones. No statistical difference was observed in HDL and tryglycerides amongst the groups. CK activity was found diminished in groups T60`, T120` and T240` as compared to the sedentary and the T30` rats. LDH was shown to be significantly diminished in the T30` group as compared to the sedentary and T60` one. Concerning paraoxonase and catalase activities and total sulphydryls no significant difference amongst the groups was found. As for free sulphydryls lower concentrations were found in T120` and T240` in relation to the other groups.

DISCUSSION

Rats that received a normoproteic diet didn't show increase in blood total proteins (Tonon et al., (2001). Pauli et al., (2003), described that moderate physical activity for a long period demands more protein than an intense activity and consequently implies in more urea production. The reason for the increase in ALP activity is still unknown bur according to Melo et al., (2004) This enzyme, in studies carried out with humans during marching activity, shows increased activity, being considered a marker of bone synthesis during physical activity practice. The experiments performed by Thomassian et al., (2007), have shown the activity of AST to be lower than in animals with better physical condition and these data differ from ours. Weigand et al., (2007) even though having done an experiment of physical exhaustion, where they state that this enzyme is used for assessing the physical conditioning of exercised animals, also didn't find increase in the activity of this enzyme. Other works also affirm that practicing extenuating activities increases up to three-fold AST activity, but according to the studies of Thomassian et al., (2007), it returns to values similar to those of resting after 30 minutes, and this do not agree with our results. ALT is released in the blood stream about 3 to 4 days after the injure and return to basal values after two weeks. According to Spinosa et al., (1999), the increase of this enzyme is related to the number of cells involved and not to the severity of the lesion.

Creatinine, since it is a produce of creatine degradation in muscle, is generally produced at a rate practically constant during physical activity, especially in when this is of long duration. Its increase leads to the conclusion that there is a degradation of muscle phosphocreatine due to the physical effort. In our experiment it could be observed that even with the increase in the volume of swimming activity creatine was augmented only after 60 min. was leads us to conclude that our protocol did not impair the renal function but, instead, it caused favorable adaptations in the animals. In relation to urea, it could be observed that its increase is accompanied by muscle proteins degradation, increasing the concentration of plasma protein as a source of energy. Lit Litvinova et al., (1989), affirm based on their studies that swimming exercise cause a significant increase in urea levels and in its urinary excretion. According to our studies swimming activity for 30 min. is followed by a return to normal values after 24 hours. Nevertheless our study differs from that of the above cited authors, since our protocol used higher volumes (120 and 240 min.) and although the sacrifice of the rats occurred after 72 hours after the last session of exercise, urea levels were kept augmented.

In studies involving physical activity it was observed that 30 min. per day are sufficient for obtaining an improvement both in physical conditioning and in the biochemical parameters. Our experiment confirms the data by Prado et al., (2002), obtained in humans, in which the effect of physical activity of low or moderate intensity is an important factor for the improvement of the levels of cholesterol and its fractions. Moghadasian (2001), states that high density lipoprotein (HDL) in rats remains in high values, and so this animals is resistant to atherosclerosis, probably due to this high HDL level, since this lipoprotein is a plasma cholesterol carrier. Physical activity was efficient in lowering the levels of VLDL and LDL cholesterol. Prado et al., (2002), have shown that the effect of physical activity is reflected in the better functioning of the processes involved in the lipid profile, that is, the increase of the activity of lipoprotein lipase, forming less particles of LDL. Some relevant factors can interfere in tryglyceride metabolism. According to Belmonte et al., (2005), low intensity physical activity has a low contribution to total oxidized tryglycerides and its contribution to total energy is small and depends on intensity, duration and level of training. Bestetti et al., (1984), report the plasma levels of tryglycerides to decrease, particularly during vigorous activity.

Sotiriadou et al., (2003), in experiments with female rats, observed a decrease in CK as well as LDH activity 72 hours after the last session of exercise. CK activity is related to LDH and these enzymes reflect cell membrane integrity, which is related to lactate formation; this substance was also not augmented, what is confirmed by our data. Besides, those authors state that CK decrease is attributed to the infiltration of phagocytic cells due to the utilization of female animals in our experiment. This suggests that estrogen plays an important role in keeping the stability of cell membrane, reducing the inflammatory response post-exercise and exerting an effect on skeletal muscle tissue and attenuating injure during exercise.

LDH catalyses the last step of glycolisis where piruvate is reduced to lactate, (Lehninger, 2002), promoting the formation of lactate. Swimming, since it is a moderate activity, did not lead to sufficient lactate formation so as to alter LDH activity and these data were confirmed by our measurement of lactate.

Paraoxonase is a protein associated mainly with high density lipoprotein (HDL) which is carried by apolipoprotein A-1 (apoA-1)and participates in the prevention of LDL oxidation Reddy et al., (2001). It is believed that it protects against atherogenic processes, besides protecting LDL from peroxidation, thus improving the reverse transportation of cholesterol. Oda et al., 2001). It is also suggested that PON protects membranes from injure caused by free radicals. Our experiment showed that there was no difference in PON activity, as well as in HDL, since the number of HDL is an important and determinant factor in PON levels; this explains PON activity. The studies by Zoppi et al., (2003), are confirmed by ours, indicating that sulphydryl activity is altered when effort intensity is higher. Besides that, they propose that unaltered total sulphydryl concentration reinforce the hypothesis of low oxidative damage. The sanme authors have also detected an increase in plasma sulphydryl groups, due to oxidative stress is induced by physical training, since low levels of oxidative stress are wished as an adaptative response of an efficient practice. Only in the volumes of 120 and 240 minutes was able to alter oxidative stress, reflecting in the decrease of free sulphydrys. According to Schneider et al., (2004), the stress is better tolerated by trained animals, what suggests an adaptation of the antioxidant systems. This leads to a better comprehension of our data, since in the trained animals in a moderate activity without overcharge and for 240 min. there was an adaptation, not leading to oxidative stress. The same authors affirm that regular training prolongs the aerobic resistance capacity and increases the antioxidative defenses.

CONCLUSION

In conclusion the present work has demonstrated that training at different volumes led to a beneficial effect on the biochemical parameters, improving the physical conditioning and metabolism. Amongst those parameters the lipid profile can be highlighted since training was able to decrease total cholesterol and its fractions from 30 minutes on. As for hepatic function a good response to training was obtained since although this was of long duration no hepatic injure was induced. The same happened with renal function, what indicates that the swimming protocol caused favorable metabolic adaptations in the animals. The LDH results confirm these adaptations since it did not cause increase in antioxidant concentrations and CK activity.

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Núcleo de Pesquisa em Ciências Biológicas – NUPEB. Departamento de Alimentos, Escola de nutrição.
Universidade Federal de Ouro Preto. Campus Universitário, Morro do Cruzeiro s/nº - 35400-000.
mesilva@enut.ufop.br, Telefone: (31) 3559-1828.

EVALUATION OF BIOCHEMICAL PARAMETERS IN FEMALE FISHER RATS SUBJECTED TO SWIMMING TRAINING IN DIFFERENT VOLUMES (30, 60, 120 AND 240 MINUTES).

ABSTRACT

In the last years various researches have stressed how much physical activity has been efficient in the prevention and treatment of diseases. In the present work we studied the biochemical and nutritional parameters which allow a general view of the benefits of exercise for the health of Fisher female rats subjected to swimming training in different volumes (30, 60, 120 and

240 minutes). Forty six animals were used which were divided into four groups with the cited volumes of exercise and one sedentary group, all fed commercial ration. Concerning the hepatic function there was an increase in total protein in groups T30`, T120` and T240`, an increase in alkaline phosphatase in group T120`, a decrease in AST in the T30` and an increase in ALT in the T240` animals. As for renal function creatinine was augmented in the T30` group in relation to the sedentary group and an increase in urea in groups T60`, T120` and T240` was observed. In lipid profile total cholesterol was found diminished in the trained animals. A decrease in creatine kinase in groups T60`, T120` and T240` and a decrease in lactic deshidrogenase in relation to the sedentary rats were also observed. Free sulphydrils were found decreased in animals of groups T120` and T240`. The present results have demonstrated the beneficial effects of training on biochemical parameters, improving the physical condition and generating a better adaptation in the LDH activity. Furthermore total cholesterol and its fractions were diminished. Good responses were also obtained in hepatic and renal functions.

KEY WORDS: training, swimming; biochemical parameters.

L´ÉVALUATION DE PARAMÈTRES BIOCHIMIQUES DANS RATAS FISCHER SOUMISES À L´ENTRAINEMENT DE NATATION DANS VOLUMES DIFFÉRENTS (30. 60. 120 et 240 MINUTES).

RÉSUMÉ

Aux années dernières, plusieurs recherches ont donné la preuve de l´efficience de l'activité physique dans la prévention de maladies. Dans ce travail, nous avons étudié les paramètres biochimiques et nutritionnels qui permettent une vision générale des avantages des exercices pour la santé de ratas Fisher. Pendant quatorze semaines, ces rats ont été soumises à l´entrainement de la natation dans des volumes différents (30, 60, 120 et 240 minutes). Ils ont été utilisés 46 rats féminins, distribuées dans 4 groupes d'exercices dans les volumes mentionnés et 1 groupe sédentaire. Tous les groupes, avec 9 animaux, recevaient la ration commerciale. Il était arrivé l´augmentation autant des protéines totales dans les groupes T30', T120' et T240' que de fosfatase alcaline dans le groupe T120'; l´une diminution de l'AST dans le groupe T30' et l´une augmentation de l'ALT dans le groupe T240'. L´augmentation de la creatinina dans groupe T30' par rapport au groupe sédentaire et une augmentation de l'uréia dans les groupes T60', T120' et T240'. Il y avait une diminution dans le cholestérol total dans les groupes qui faisait des exercices autour de 30`. Une diminution de la creatina quinase dans les groupes T60', T120' et T240' par rapport aux groupes T30' et sédentaire. Une diminution de la desidrogenase làctica (LDH) du groupe T30' par rapport au groupe sédentaire et une diminution dans la sulfidrila libèrée des groupes T120' et T240'. L'étude a montré que l'effet de l´entrainement sur les paramètres biochimiques a amélioré le conditionnement physique, en produisant de meilleures adaptations dans les résultats de l´LDH, sans promouvoir l'augmentation des antioxydants et de la creatina quinase. Encore, il a mis en evidence l´efficace de l'activité physique dans la réduction des concentrations du cholestérol total et de leur sous-fractions. De bonnes réponses à l´entrainement ont été aussi observées dans les protéines totales, fosfatase, ALT, AST, creatinina et uréia.

MOTS-CLÉ: entrainement, natation, paramètres biochimiques.

EVALUACIÓN PARÁMETROS BIOQUÍMICOS EN RATAS FISCHER SOMETIDOS A ENTRENAMIENTO DE NATACIÓN EN DIFERENTES VOLÚMENES (30, 60, 120 Y 240 MINUTOS).

RESUMEN

En los últimos años varias pesquisas han evidenciado cuanto la actividad física es eficiente en la prevención y el tratamiento de enfermedades. En este trabajo hemos estudiado los parámetros bioquímicos y nutricionales que permiten una visión general de los beneficios del ejercicio para la salud de ratas Fisher hembras sometidas al entrenamiento de natación en diferentes volúmenes (30, 60, 120 y 240 minutos) por catorce semanas. Fueron utilizadas 46 ratas hembras distribuidas en seis grupos con los volúmenes de ejercicio citado arriba, todos con nueve animales recibiendo ración comercial. En la función hepática hubo aumento en las proteínas totales en los grupos T30`, T120` y T240`, aumento en la fosfatasa alcalina en el T120`, disminución de la AST en el T30` y aumento de la ALT en los animales del grupo T240`. En la función renal hubo aumento en la creatinina en el grupo T30` en relación al grupo sedentario y un aumento de la urea en los grupos T60`, T120` y T240`. El perfil lipídico mostró disminución del colesterol total en todos los grupos entrenados. Disminución de la creatina quinasa en los grupos T60`, T120` y T240` en relación a los grupos T30` y sedentario y disminución de la desidrogenasa láctica han también sido también observadas. Hubo disminución de las sulfidrilas libres en los grupos T120` y T240`. El presente estudio demostró que el efecto del entrenamiento sobre parámetros bioquímicos mejoró el condicionamiento físico, generando mejores adaptaciones el los resultados de LDH, sin promover aumento de los antioxidantes y de la creatina quinasa, siendo eficiente en disminuir las concentraciones de colesterol total y sus fracciones. Buenas despostas al entrenamiento fueron también observadas en la funciones hepática y renal.

PALABRAS-LLAVE: Entrenamiento; natación; parámetros bioquímicos.

AVALIAÇÃO DE PARÂMETROS BIOQUÍMICOS EM RATAS FISCHER SUBMETIDAS AO TREINAMENTO DE NATAÇÃO EM DIFERENTES VOLUMES (30, 60, 120 e 240 MINUTOS).

RESUMO

Nos últimos anos, várias pesquisas têm evidenciado o quanto a atividade física tem sido eficiente no tratamento e prevenção de doenças. Neste trabalho estudamos os parâmetros bioquímicos e nutricionais que permitem uma visão geral dos benefícios dos exercícios para a saúde de ratas Fisher submetidas ao treinamento de natação em diferentes volumes (30, 60, 120 e 240 minutos) por quatorze semanas. Foram utilizadas 46 ratas, distribuídas em 4 grupos de exercícios nos volumes citados e 1 grupo sedentário, todos com 9 animais, recebendo ração comercial. Na função hepática houve um aumento nas proteínas totais nos grupos T30`, T120` e T240`, aumento da fosfatase alcalina no grupo T120`, uma diminuição da AST do grupo T30` minutos e um aumento da ALT no grupo de 240 minutos. Na função renal houve um aumento da creatinina do grupo T30` em relação ao grupo sedentário e um aumento da uréia dos grupos T60`, T120` e T240`. No perfil lipídico houve uma diminuição no colesterol total nos grupos que treinaram a partir dos 30 minutos. Uma diminuição da creatina quinase dos grupos T60`, T120` e T240` em relação aos grupos T30` e ao sedentário e uma diminuição da desidrogenase láctica (LDH) do grupo T30` em relação ao grupo sedentário foram também observados. Houve uma diminuição nas sulfidrilas livres dos grupos T120` e T240`. O presente estudo demonstrou que o efeito do treinamento sobre os parâmetros bioquímicos melhorou o condicionamento físico, gerando melhores adaptações nos resultados da LDH, sem promover aumento dos antioxidantes e da creatina quinase, sendo eficiente em diminuir as concentrações do colesterol total e de suas subfrações. Boas respostas ao treinamento foram também observadas nas funções hepática e renal.

PALAVRAS-CHAVE: Treinamento, natação, parâmetros bioquímicos.

PUBLICAÇÃO NO FIEP BULLETIN ON-LINE: http://www.fiepbulletin.net/80/a1/32