81 - STUDY OF THE HEPATIC FUNCTION OF EXERCISED AND NUTRITIONALLY RECOVERED ANIMALS

EMERSON CRUZ DE OLIVEIRA; RINALDO CARDOSO DOS SANTOS;
MARIA LÚCIA PEDROSA; MARCELO EUSTÁQUIO SILVA.
UNIVERSIDE FEDERAL DE OURO PRETO, OURO PRETO - MG, BRASIL
marcelsil@gmail.com

INTRODUCTION

Malnourished humans under treatment present a set of clinical, biochemical and histological manifestations that may characterize the so called Nutritional Recovery Syndrome (NRS). Hepatomegalia is the main clinical manifestation, affecting 79% of the malnourished and 94% of the kwashiorkor cases. It begins around the 20th day of treatment reaching the maximum at the 35th day, seeming not to correspond to steatosis, since this is the largest phase of malnutrition and it diminishes as long as NRS takes place, resulting in higher deposition of fat in the hepatocyte. Disproteinemia is the most observed manifestation. The decreased level of total protein, albumin and betaglobulin is reverted during NRS. On the other hand the gammaglobulin fraction which is almost always augmented, increases even more, reaching hypergammaglobulinemia during NRS. The more evident histological manifestation occurs in liver and before this organ returns to normality some alterations can be observed: the disappearance of steatosis is gradual and hepatic tissue shows a condition characterized by an increase in cell volume, reduced number of intraprotoplasmatic granula and increase in absolute quantities of glycogen, water and nitrogen. This histological change would be responsible for hepatomegalia (Marcondes et al., 1976).

Ashworth (1969) studied nutritional recovery in eight children aging from 10 months to 3 years who had body weight varying from 50 to 70% of normal weight for height. During nutritional recovery the growth rate was 15-fold higher than normal children with same age and 5-fold than that of children with similar height and weight. After reaching normal weight for height the children's food ingestion dropped to a level similar to that of normal counterparts with same height and weight, but younger.

We recently observed that animals in nutrition recovery showed weight gain approximately twice as high as eutrofic ones and ten times higher than the malnourished ones. It was also possible to observe important alterations in lipid profile of the malnourished animals which were not influenced by exercise (Oliveira et al., 2007).

As liver plays a central role in carbohydrate, protein and lipid metabolism we decided to evaluate this organ in trained animals submitted to protein-calorie malnutrition followed by nutrition rehabilitation.

OBJECTIVE

To evaluate the effect of low volume and intensity training on the hepatic function of female rats submitted to post-weaning protein-calorie malnutrition and to nutritional recovery.

MATERIAL AND METHODS

Animals

Fifty two female Fisher rats were divided into six groups: Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS), Recovered Trained (RT), Malnourished Sedentary (MS) and Malnourished Trained (MT). Animals received filtered water and food *ad libitum* and were kept in a room with dark/light cycle of twelve hours and temperature 25 ± 1 ºC.

Diets and malnutrition protocol

During the experiment diet AIN-93M (Reeves et al., 1993) was used, with modifications in protein concentration (Table I). Right after weaning, at 28 days of age animals of the malnourished group received non-protein diet for 30 days while control one was given AIN-93M diet. After this the malnourished group was divided into two. Half received a low protein (6% casein) diet and the others received the control diet and this was the recovered group. Concomitantly all groups were equally divided into trained and sedentary.

Table I. Composition of diet in g/1000g of diet.

Composition/Diets	Non-protein	Low protein	Control AIN-93M
Casein	0.0	60.0	140.0
Oil	40.0	40.0	40.0
Vitamin mixture[2]	10.0	10.0	10.0
Salt mixture[1]	35.0	35.0	35.0
Fiber	50.0	50.0	50.0
Choline	2.5	2.5	2.5
Corn Starch	862.5	802.5	722.5

[1]Vitamin mixture (g/Kg de mistura): Retinol acetate - 2.000.000IU / Cholecalciferol - 200.000IU / p-aminobenzoic acid - 10.00 / I-Inositol - 10,00 / Niacin - 4.00 / Calcium Pantotenate - 4.00 / Riboflavine - 0.80/ Tiamine HCL - 0.50 / Piridoxine HCL - 0.50 / Folic acid - 0.20 / Biotin - 0.04 / Vitamin B12 - 0.003 / Sucrose - q.s.p. 1000. / Choline - 200.0 / -Tocopherol - 10.000IU [2]Salt mixture (g/kg of mixture): NaCl - 139.3 / KI - 0.79 / MgSO4.7H2O - 57.3 / CaCO3 - 381.4 / MnSO4.H2O - 4.01 / FeSO4.7H2O - 27.0 / ZnSO4.7H2O - 0.548 / CuSO4.5H2O - 0.477 / CoCl2.6H2O - 0.023 / KH2PO4 - 389.0.

Training

Exercised animals were initially adapted to water at 31ºC ± 1 ºC in the as follows: First and second days, 30 min. in a shallow pool. Third and fourth days, two series of 15 min by 5 min. interval in a pool 50 cm deep and in the fifth day they swum 30 min continuously in this same depth. From the second to the ninth week exercised animals repeated the session of the fifth day of adaptation, 5 days/week. Sedentary animals were submitted to contact with water during 30 min. in a shallow pool during the whole experiment in order to undergo the same handling stress.

Nutritional and biochemical evaluation

The control of food ingestion was done during the last three weeks of experiment. After nine weeks animals were sacrificed 48 hours after the last session of exercise and 8 hours of fasting. Blood was collected and immediately centrifuged for serum separation. Biochemical determinations were performed using laboratory Labtest Diagnóstica kits according to manufacturer's instructions. After blood collecting abdomens were opened for liver separation and weighing.

Statistical analysis

Comparison among groups was done by two-way ANOVA ($p<0.05$) followed by *post hoc* Bonferroni.

RESULTS

Body weight, food ingestion and liver weight

The higher body weight of control animals was an expected result as a function of their nutritional status. We also expected that the values of animals in groups RS and RT were statistically similar to those in groups CS and CT, but this was only partially observed since values of the RT animals were slightly lower than RS. If only body weight is considered it can be noted that RS animals achieved nutritional recovery. Nevertheless the effect of exercise on RT rats should not be taken as a bad one once statistically this group was equal to group RS. Malnourished animals presented a different response in relation to the others and in this case, although statistically equal, average values of the exercised animals were higher than those of the sedentary ones (Table II).

Table II - Body weight, Food Ingestion (FI), Liver weight of groups: Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS), Recovered Trained (RT), Malnourished Sedentary (MS) and Malnourished Trained (MT) after nine weeks of experiment.

Groups\Parameters	Body Weight(g)	FI (g)	Liver (g)
CS	206.83 ± 13.68^{a}	106.77 ± 6.17	5.87 ± 0.52^{a}
CT	201.17 ± 13.18 ^{a}	117.51 ± 7.91	5.79 ± 0.55^{a}
RS	186.17 ± 15.38^{a,b}	95.06 ± 8.80	5.84 ± 0.45^{a}
RT	170.29 ± 7.18^{b}	85.02 ± 8.42	4.84 ± 0.46^{b}
MS	55.14 ± 16.31^{c}	68.37 ± 26.97	2.00 ± 0.61^{d}
MT	74.14 ± 19.63^{c}	62.38 ± 23.76	3.04 ± 0.66^{c}
P value (Anova Two Way)			
Nutritional Status	<0.0001	<0.0001	<0.0001
Training	NS	NS	NS
Interaction	0.0080	NS	<0.0001

Results are expressed as mean ± standard deviation. NS = non significant. Different letters indicate significative difference $p < 0.05$.

In relation to food ingestion there was a significant difference as a function of the nutritional status. Control animals exhibited the higher values followed by the recovered and the malnourished ones. No significant difference was observed due to training nor any interaction between nutritional status and training (Table II).

In the case of liver weight there was a significant difference due to nutritional status. Control rats had the higher values followed by recovered ones while malnourished groups showed the lower values. No significant difference was noted as a function of training. However there was an interaction between nutritional status and training for liver weight: CS, CT and RS were similar to each other and showed higher values as compared to the other ones. RT animals were also different from MT ones and these by their turn were different from MT which had the lowest values (Table II).

Hepatic Function

Serum concentration of total proteins revealed a significant difference due to the nutritional status. Recovered rats showed the highest values followed by recovered and malnourished ones respectively. No significant difference was observed due to the training protocol. However there was an interaction between nutritional status and training: RT, CS and RS animals had the highest values followed by CT ones which were similar to group RS and the lowest values were found in groups MS and MT (Table III).

In relation to average serum concentration of albumin all groups were statistically similar to each other. For the globulins concentration the nutritional status influenced the results: the recovered animals had the highest values followed by the control ones while the malnourished rats exhibited the lowest numbers. There was no significant difference due to training but an interaction between this and the nutritional status was observed: RT, RS and CS animals had the highest values, followed by CT while in those of groups MT and MS globulins concentration was lower (Table III).

Table III - Average serum concentration of Total Proteins, Albumin and Globulins of groups Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS), Recovered Trained (RT), Malnourished Sedentary (MS) and Malnourished Trained (MT) after nine weeks of experiment

Groups\ Parameters	Total proteins (g/L)	Albumin (imol/L)	Globulins (g/dL)
CS	69.75 ± 3.75^{a}	491.54 ± 74.47	3.58 ± 0.41^{a,b}
CT	63.64 ± 4.74^{b}	487.89 ± 57.8	3.00 ± 0.33^{b}
RS	69.72 ± 3.68^{a,b}	488.95 ± 9.99	3.60 ± 0.38^{a,b}
RT	71.37 ± 4.04^{a}	489.65 ± 34.75	3.76 ± 0.39^{a}
DS	60.90 ± 4.78^{c}	520.45 ± 31.31	2.50 ± 0.29^{c}
DT	60.34 ± 5.26^{c}	496.10 ± 69.52	2.61 ± 0.70^{c}
P value (Anova Two Way)			
Nutritional Status	<0.0001	NS	<0.0001
Training	NS	NS	NS
Interaction	0.0299	NS	0.0151

Results are expressed as mean ± standard deviation. NS = non significant. Different letters indicate significative difference $p < 0.05$.

ALT activity was influenced by the nutritional status. Malnourished rats had the highest values followed by controls and recovered ones respectively. A significant difference due to the training protocol was seen: numbers amongst the trained animals were superior to those in the sedentary groups. No interaction between nutritional status and physical activity was observed (Table IV).

Table IV - Average activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) of groups Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS), Recovered Trained

(RT), Malnourished Sedentary (MS) and Malnourished Trained (MT) after nine weeks of experiment

Groups\Parameters	ALT (U/mL)	AST (U/mL)	ALP (iKat/L)
CS	20.16 ± 7.03	65.20 ± 6.95^{b}	15.71 ± 3.99^{d}
CT	21.79 ± 8.05	63.11 ± 9.75^{b}	18.93 ± 5.64^{c,d}
RS	7.96 ± 2.97	25.04 ± 5.96^{c}	35.20 ± 11.42^{b}
RT	11.11 ± 3. 22	28.81 ± 5.15^{c}	26.76 ± 6.06^{b,c}
DS	23.29 ± 5.05	63.03 ± 10.43^{b}	47.35 ± 7.68^{a}
DT	36.15 ± 14.45	99.28 ± 13.16^{a}	56.20 ± 6.13^{a}
	P value (Anova Two Way)		
Nutritional Status	<0.0001	<0.0001	<0.0001
Training	0.0154	<0.0001	NS
Interaction	NS	<0.0001	0.0085

Results are expressed as mean ± standard deviation. NS = non significant. Different letters indicate significative difference $p < 0.05$.

The nutritional status influenced AST activity too. In the malnourished animals the enzyme was more active while controls had intermediate values followed by the recovered ones. A significant difference was shown between sedentary and exercised animals, with higher values due to the physical activity. There was also an interaction between training and nutrition: MT animals had the highest values followed by the CS, CT and MS groups. RT and RS rats had values similar to each other and lower than control and recovered ones (Table IV).

Alkaline phosphatase activity was also influenced by the nutritional status with malnourished animals exhibiting the highest values followed by recovered and control ones respectively. On the other hand physical exercise had no influence on this parameter. Nevertheless an interaction between nutrition and exercise could be identified as far as MT and MS animals had the highest values followed by RS which was similar to RT. This group by its turn, was statistically similar to CT and this was equal to CS. The sedentary control group exhibited the lowest values (Table IV).

DISCUSSION

The higher food ingestion in the control group, followed by recovered and malnourished ones is in accordance with the results of Hansen-Smith et al. (1977) for control and malnourished animals, both sedentary during a period of eight weeks but not with those obtained by Oldfors & Sourander (1985) who observed a higher food ingestion in the control animals in the four initial weeks and equal ingestion between control and malnourished animals, both sedentary and exercised at the end of the experiment (12 weeks). Some authors (Ocken & Grunewald, 1988; Papoti et al., 2003) did not find difference between control and recovered animals in food ingestion.

Recovered animals had higher values of serum total proteins than the others and it has been described that nutrition recovery causes a faster growth (Ashworth, 1969), what makes us believe that the metabolic rate in the recovering animals might be augmented; it is possible to observe that there is a relationship between the increase in total proteins which is generated by an increase in the globulin fraction, possibly gammaglobulins (antibodies) and this would be the explanation for the augmented spleen of these animals (data not shown). In the malnourished group the levels of albumin that were found can only be explained by the reduction of the globulin fraction in these animals. This response of the rehabilitated animals did not occur in another work (Silva et al., 1999), but the absence of a significant difference in the serum concentration of our study reproduced that obtained by them. In the present work we calculated the concentration of globulins through the difference between total protein and albumin for each animal. Silva et al. (1999) did not calculate the difference this way; so taking the average values presented by them it was possible to come to an estimate of the globulin concentration in that paper. We observed that the absence of difference in the albumin fraction in both works is due to the decrease in globulins following the observed difference in total protein.

The lower activities of AST and ALT in the recovered group in relation to controls is due to an increase in protein synthesis since the animals were leaving a malnutrition condition and receiving more protein in the diet. As those enzymes are needed in the process of protein degradation their activities are expected to be reduced when anabolism is overcoming catabolism. In the malnourished group which is still receiving a low protein diet the enzymes activities is augmented above control values; this shows that the malnutrition protocol even in the phase of maintenance of malnutrition causes strong catabolism. The trained animals showed increased activity of both enzymes and this fact can be noted especially in the animals of group MT. We believe that this occurs due to a higher mobilization of fat from the adipocyte to the liver, what could explain the heavier livers in this group. The organ might not manage to export stored fat due to the lack of lipoproteins and fat transporting enzymes. Koutedakis et al. (1993) comparing trained and not trained individuals verified that exercise increases in an acute manner the activities of AST and ALT and the level of fitness and exercise duration correlate with the activities of these enzymes. Amelink et al. (1988) found the AST activity to be augmented in 1% in male rats right after a running session but the same did not happen to the females what indicates that modifications in this enzyme appear to have to do with gender. In the present experiment we did not aim at analyzing the acute effects of exercise on the activities of the two enzymes but rather if training and the nutritional status would affect in a chronic mode the enzyme activity in the three nutritional conditions. Thus the rats were sacrificed 48 hours after the last exercise session. So what can be observed is the effect of nine-week training and that of the nutritional status on the activities of the two enzymes.

It was possible to furthermore note that malnutrition implied an increase in AST and in ALT as well as in alkaline phosphatase and that nutritional rehabilitation tended to revert the increase in ALP. The ALP values were higher in the malnourished animals and were reduced by rehabilitation. Nevertheless this reduction was not sufficient to bring the values of the rehabilitated animals closer to those of the controls. ALP is an enzyme located in the membrane of liver cells and can indicate cell lysis provided its concentration is augmented in plasma. Differently from AST and ALT which are involved in protein degradation and are diminished during protein synthesis, ALP activity was augmented in the recovered animals. Since malnourished animals were receiving a diet containing 6% protein it was not possible to occur protein synthesis in these animals as shown by recovered ones which were given 12%. Thus the values of AST, ALT and ALP allow us to conclude that the malnourished animals suffered hepatic injure.

CONCLUSION

The nutrition rehabilitation protocol was efficient in promoting the recovery of body weight of the recovered sedentary rats but training did not alter this situation. Considering ALT, AST and ALP activities and the concentration of globulins, data suggest hepatic injure in the malnourished animals but not in the recovered ones.

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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/n°-354000-000.
marcelsil@gmail.com

STUDY OF THE HEPATIC FUNCTION OF EXERCISED AND NUTRITIONALLY RECOVERED ANIMALS

ABSTRACT

The goal of the present work was to study the hepatic function of animals in a process of nutritional recovery associated or not with physical exercise (swimming 30 minutes/day during 8 weeks). Fifty two female Fisher rats were divided into six groups: Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS), Recovered Trained (RT), Malnourished Sedentary (MS) and Malnourished Trained (MT). We have concluded that the protocol of nutritional recovery was efficient in promoting the recovery of body weight of the animals RS but exercise did not change this picture. Considering the activities of aminotransferases, alkaline phosphatase and the concentration of globulins, data suggest hepatic injure in the malnourished animals, and this has not occurred with recovered ones.

KEY WORDS: Malnutrition, exercise, hepatic function.

ÉTUDE DE LA FONCTION HÉPATIQUE DES ANIMAUX EXERCITÉS ET DES QUI SONT EN PROCÈS DE RÉTABLISSEMENT ALIMENTAIRE

RÉSUMÉ

L'objectif de cette recherche a été celui d'étudier la fonction hépatique des animaux qui sont en procès de rétablissement alimentaire, associé ou non-associé aux exercices physiques (natation 30 minutes chaque jour, 5 jours par semaine pendant 8 semaines). On a fait l'expérience avec 52 rats Fisher, separées dans 6 groups: Contrôle Sédentaire (VS), Contrôle Entraîné (CE), Guéri Sédentaire (GS), Guéri Entraîné (GE), Sous-Alimenté Sédentaire (SS) e Sous-Alimenté Entraîné (SE). On a conclu que le protocole de rétablissement alimentaire a été efficace pour faire avancer le rétablissement du poids des animaux GS, mais l'entraînement n'a pas modifié ce quadre. En tenant compte les activités de l'aminotransferases, de la fosfatase alcaline aussi que des concentrations des globulines, les rapports suggèrent un péjudice hépatique chez les animaux sous-alimentés, ce qu'on ne voit pas chez les animaux guéris.

MOTS-CLÉS: sous-alimenté, exercices physiques, fonction hépatique.

ESTUDIO DE LA FUNCIÓN HEPÁTICA DE ANIMALES EJERCITADOS Y RECUPERADOS NUTRICIONALMENTE

RESUMEN

El objetivo de este trabajo fue estudiar la función hepática de animales en proceso de recuperación nutricional, asociada o no al ejercicio físico (natación 30 minutos por día durante 8 semanas). Fueron utilizadas 52 ratas Fischer hembras, distribuidas en seis grupos: Control Sedentario (CS), Control Entrenado (CE), Recuperado Sedentario (RS) Recuperado Entrenado (RE), Desnutrido Sedentario (DS) y Desnutrido Entrenado (DE). Hemos concluido que el protocolo de recuperación nutricional fue eficiente en promover la recuperación del peso de los animales RS pero el entrenamiento no alteró este cuadro. Considerando las actividades de las aminotransferasas, fosfatasa alcalina y las concentraciones de las globulinas, los datos son sugerentes de comprometimiento hepático en los animales desnutridos, facto que no ha pasado con los recuperados.

PALABRAS-LLAVE: Desnutrición, ejercicio, función hepática.

ESTUDO DA FUNÇÃO HEPÁTICA DE ANIMAIS EXERCITADOS E EM PROCESSO DE RECUPERAÇÃO NUTRICIONAL

RESUMO

O objetivo desse trabalho foi estudar a função hepática de animais em processo de recuperação nutricional, associada, ou não, ao exercício físico (natação 30 minutos por dia, 5 dias por semana durante 8 semanas). Foram utilizadas 52 ratas Fisher, distribuídas em seis grupos: Controle Sedentário (CS), Controle Treinado (CT), Recuperado Sedentário (RS); Recuperado Treinado (RT); Desnutrido Sedentário (DS) e Desnutrido Treinado (DT). Concluímos que o protocolo de recuperação nutricional foi eficiente em promover a recuperação do peso dos animais RS, mas o treinamento não alterou esse quadro. Considerando as atividades da aminotransferases, fosfatase alcalina e concentrações das globulinas, os dados sugerem um comprometimento hepático nos animais desnutridos, fato que não ocorreu nos animais recuperados.

PALAVRAS-CHAVE: Desnutrição, exercício, função hepática.