26 - EFFECTS OF EARLY PROTEIN CALORIE MALNUTRITION AND OF NUTRITIONAL RECOVERY ON BIOCHEMICAL PARAMETERS IN FEMALE RATS SUBMITTED TO SWIMMING TRAINING

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INTRODUCTION

Plasma or total proteins are involved in multiple functions such as maintenance of the osmotic pressure and blood viscosity, besides nutrient, metabolites, hormones and excretion products transport (Guyton, 2002). Normally total protein concentration is found augmented in dehydration (hemoconcentration), in rheumatoid arthritis and in chronic infections. On the other hand diminished concentrations are found when hepatic failures Intestinal and renal disorders hemorrhage and serious malnutrition occur (Wilkinson & Mendehall, 1963).

Albumin is the most abundant plasma protein accounting for 50% of the total but its concentration can decrease (hypoalbuminemia) in various situations such as those cited above. When albumin levels are diminished and that of globulins (particularly gamma-globulins) is augmented (Havens, Jr. *et al*., 1954), we can affirm that a hepatic disease is established, primarily due to a diminished synthesis of albumin (Wilkinson & Mendehall, 1963).

We have recently studied hepatic function in female rats submitted to post-weanling protein calorie malnutrition associated or not to physical exercise (Oliveira *et al*., 2008). In that experiment we used the AIN-93M diet (Reeves *et al.*, 1993) modifying the protein concentration in order to obtain a non protein diet (0% casein) and a low protein one (6% casein). The malnutrition protocol consisted of offering to the malnourished animals the non protein diet during 30 days, just after weanling, that is at 28 days of age, while the control group received the AIN-93M diet. Then the malnourished group was divided into two: one half was kept in the low protein diet while the other was offered received the control one, making this the recovered group. Concomitantly the animals of all groups were divided into trained and sedentary ones, making the 6 groups of that work. Among other things it was possible that for body weight only the rats of the recovered sedentary group reached a nutritional recovery. Nevertheless the effect of exercise in the animals of the recovered trained animals could not be considered as bad since statistically this group was equal to the recovered sedentary. In relation to the average food ingestion and serum total protein concentration the control animals presented higher values than those of the recovered group.

In another study using the same experimental groups and the same malnutrition protocol described above we observed that the recovering rats showed two-fold and ten-fold increases in weight gain as compared respectively to the eutrophic and malnourished ones (Oliveira *et al*., 2007).

In the present study the malnutrition protocol was modified in order to have animals recovering from early malnutrition, a process that we believed to leave more pronounced sequels than those found in the former experiments.

OBJECTIVE

The objective of the present work was to study nutritional and biochemical parameters of female rats submitted to protein calorie malnutrition after birth and subsequent nutritional recovery, associated or not with physical exercise.

METHODOLOGY

Animals

Thirty two female Fisher rats were divided into four groups: Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS) and Recovered Trained (RT). 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 the AIN-93M diet was used (Reeves *et al*., 1993) with protein contents being modified (Table I). The pregnant animals were monitored during pregnancy and in the day of delivery they were divided into two groups. The first one was given the low protein diet (malnourished group) while to the second the AIN-93M diet was offered (control group). The mothers received these diets until the pups were 28 days old when weanling was done. Then the malnourished group was moved to the control diet, thus becoming the recovered group. Concomitantly all groups were divided into trained and sedentary.

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

Composition/Diets	Control (AIN-93M)	Low protein
Casein	160.0	67.0
Oil	40.0	40.0
Vitamin mixture[1]	10.0	10.0
Salt mixture[2]	35.0	35.0
Fiber	50.0	50.0
Choline	2.5	2.5
Corn Starch	702.5	795.5

[1]Vitamin mixture (g/Kg de mixture): 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 eighth 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 hole experiment in order to undergo the same handling stress.

Nutritional and biochemical evaluation

Food ingestion was measured by recording, for each cage the amount of food offered to the animal, the food left and that wasted which was collected in trays placed below the cage.

After one week of adaptation and eight weeks of training the animals were weighed, pre- anesthetized with ether and sacrificed by exsanguination approximately 72 hours after the last training session and after 8 hours of fasting. Each cage was carefully inspectioned and food and trays were removed in order to ensure that the rats had no access even to feces during the fasting period to avoid coprophagy. The skin of the animals was cut next to the front limb to allow the localization of the brachial plexus. Blood was removed from this plexus and then centrifuged at 3000 rpm for 15 minutes for obtaining serum and plasma; these were kept under refrigeration (-4ºC). All dosages were performed up to four days after sacrifice except hemoglobin that was measured immediately after blood collection using 10 ìL of blood. Biochemical determinations were performed using Labtest kits according the manufacturer's instructions.

Statistical treatment of data

The comparison between groups was done using *Two-way ANOVA* and differences were considered statistically significant for $p<0.05$. This analysis allows us to affirm whether there were differences as a function of the nutritional status or training and if both had influence on each other, the so called interaction. When interactions were found the Bonferroni *post hoc* was also applied to stress the differences for $p<0.05$ and to allow that different letters were attributed to different groups in a same column of a given parameter.

RESULTS AND DISCUSSION

Body weight, weight gain and food ingestion

The heavier bodies of SC and TC animals in the beginning of the training period was an expected result as a function of the malnutrition protocol employed in the first part of the experiment. SR and TR rats that in the beginning of the experiment had approximately 30% of the body weight of SC and TC increased this figure to 70%, what was also expected. But due to the severity of the malnutrition process imposed to the SR and ST animals a complete recovery with statistical equivalency between recovered and control animals would be difficult to be reached, differently from what had been formerly observed by us when malnutrition was imposed by the time of weanling (Oliveira et al., 2008)

Food ingestion (FI) showed that training generates higher food consumption without however leading to a significant weight gain even among the animals in the recovery process in the same period (Table II). The higher weight gain of the control animals opposes former results from our laboratory for another malnutrition protocol (Oliveira et al., 2007) as well as results observed in children in nutritional rehabilitation (Ashworth, 1969). Other authors (Ocken & Grunewald, 1988; Papoti *et al*., 2003) did not find differences between control and recovered groups for food ingestion.

In the specific case of food ingestion the interaction found between nutritional status and training allows us to conclude that the increase of food ingestion imposed by the employed training is not influenced by nutritional status, the significant increase occurred in TC animals in relation to SC was not followed by TR rats when compared with SR ones (Table II).

Table II Initial weight, final weight, weight gain and food ingestion (FI) of groups: Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS) and Recovered Trained (RT).

Groups\Parameters	Initial Weight (g)*	Final Weight (g)	weight gain (g)#	FI (g) #
SC (n=8)	53.13 ÿ 10,47	151.25 ÿ 18.51	19.50 ÿ 5.76	87.46 ÿ 23.58[c]
TC (n=8)	53.00 ÿ 10,52	155.13 ÿ 13.91	18.63 ÿ 6.05	156.85 ÿ 21.38[a]
SR (n=8)	18.75 ÿ 3,15	108.75 ÿ 11.21	17.75 ÿ 5.60	118.93 ÿ 20.18[b]
TR (n=8)	18.38 ÿ 2,97	107.00 ÿ 13.82	17.38 ÿ 3.07	125.81 ÿ 11.22[b]
p value (Anova Two Way)				
Nutritional Status	P < 0.05	P < 0.05	NS	NS
Training	NS	NS	NS	P < 0.05
Interaction	NS	NS	NS	P < 0.05

Results are expressed as mean ± standard deviation. NS = non significant. Different letters indicate significant difference in interaction ($p < 0.05$). *Initial weight in the training start. # Weight gain and food ingestion of two weeks.

Biochemical evaluation

The serum concentrations of total proteins and albumin (Table III) show the sensitivity of these indicators of nutritional status for identifying organisms recovering from malnutrition. As animals SR and TR received a diet with protein concentrations below their needs during the first 28 days of life, sequels of the deficiency remain after 90 days of age, what shows the severity of the malnutrition protocol and the applicability of these parameters for evaluating it. The absence of difference for globulins shows that albumin, as the major fraction of serum proteins may have been responsible for the lower value of total proteins in the recovering animals (Table III). Since a higher weight gain in these was not observed we believe that higher values of total proteins in the control animals is a normal result, although it opposes our former results using other malnutrition protocol (Oliveira et al., 2008).

Table III Average serum concentration of total proteins, albumin and globulins of groups Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS) and Recovered Trained (RT).3.11 ± 0.30 3.20 ± 0.68

Groups\Parameters	Total Proteins (g/dL)	Albumin (g/dL)	Globulins (g/dL)*
SC (n=8)	7.03 ÿ 0.33	3.30 ÿ 0.22	3.73 ÿ 0.27
TC (n=8)	6.66 ÿ 0.32	3.27 ÿ 0.23	3.39 ÿ 0.32
SR (n=8)	6.37 ÿ 0.23	2.94 ÿ 0.16	3.43 0.29
TR (n=8)	6.31 ÿ 0.77	3.11 ÿ 0.30	3.20 ÿ 0.68
p value (Anova Two Way)			
Nutritional Status	P < 0.05	P < 0.05	NS
Training	NS	NS	NS
Interaction	NS	NS	NS

Results are expressed as mean ± standard deviation. NS = non significant. Different letters indicate significant difference $p < 0.05$. *Total proteins albumin = globulins.

Glycemia when measured right after exercise is found lowered both in experimental animals and in humans (Farrell *et al*., 1991; Oakes *et al*., 1997; Hernandez *et al*., 2000). Nevertheless when the chronic effect of exercise is considered a reduction of blood glucose is not observed in animals (Cunha et al., 2005) or in humans (Banz et al., 2003), both well nourished. Our goal was to study only the chronic effect of exercise and we observed for serum glucose concentration an interaction between nutritional status and training what indicates that the former is influenced by and influences the result of the later. The statistically significant increase found in SR animals in relation to TR was not seen in SC as compared to TC. On the contrary a tendency to the inverse result was observed, that is, animals in group TC presented higher values than SC, although without significant difference. Faria et al., (2007) carried on a study aiming at evaluating if the volume of work could interfere in glucose and hemoglobin concentrations in female rats. They used a group of sedentary animals and groups that swam 30, 60, 120 and 240 minutes for 14 weeks. Only those swimming 240 minutes showed significant increases in relation to the sedentary ones. In the same study, the concentration of hemoglobin was also found augmented in the animals that swam more than 60 minutes in relation to the sedentary ones.

Table IV Average serum concentration of hemoglobin and glucose of groups Control Sedentary (CS), Control Trained (CT), Recovered Sedentary (RS) and Recovered Trained (RT).

Groups\Parameters	Hemoglobin (g/dL)	Glucose (mg/dL)
SC (n=8)	16.09 ÿ 1.03	79.67 ÿ 12.18[b]
TC (n=8)	16.12 ÿ 1.81	107.33 ÿ 20.01[a,b]
SR (n=8)	16.64 ÿ 1.67	113.17 ÿ 15.63[a]
TR (n=8)	14.88 ÿ 0.44	81.33 ÿ 18.05[b]
p value (Anova Two Way)		
Nutritional Status	NS	NS
Training	NS	NS
Interaction	NS	P < 0.05

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

CONCLUSION

The evaluation of biochemical and nutritional parameters showed that the animals did not present a nutritional rehabilitation in the studied period and that the applied training was not capable of interfering in this process. Training generated a higher food intake without leading to a significant weight gain even in the recovering animals. The interaction between nutritional status and training for food ingestion and serum glucose concentration did not follow a similar pattern what indicates the need for studying the present model but using other indicators of carbohydrate metabolism such as, for instance a glucose tolerance curve besides other exercise models or even more intense exercise or with a higher volume of work.

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EFFECTS OF EARLY PROTEIN CALORIE MALNUTRITION AND OF NUTRITIONAL RECOVERY ON BIOCHEMICAL PARAMETERS IN FEMALE RATS SUBMITTED TO SWIMMING TRAINING

ABSTRACT

The objective of the present work was to study nutritional and biochemical parameters of female rats submitted to protein calorie malnutrition after birth and subsequent nutritional recovery, associated or not with physical exercise (swimming 30 minutes/day, 5 days/week during 8 weeks). 32 female Fisher rats were used which were divided into four groups: Sedentary Control (SC), Trained Control TC), Sedentary Recovered (SR) and Trained Recovered (TR). Food ingestion, weight gain and serum concentrations of total protein, albumin, hemoglobin and glucose were studied. It was observed that training produced increased food ingestion but without significant weight gain. Biochemical evaluation showed that the animals did not present nutritional recovery in the studied period and that training did not interfere in this process. The interaction between nutritional status and training for both food ingestion and serum glucose concentration signals the necessity of studying the present using other indicators of carbohydrate metabolism, for instance a glucose tolerance curve, besides of course other exercise models and even more intense exercise or a higher work volume.

KEYWORDS: malnutrition, nutritional recovery, exercise. **EFFETS DE LA MALNUTRITION PROTEIQUE-CALORIQUE PRECOCE E DE LA RECUPERATION NUTRITIONNELLE SUR LES PARAMETRES BIOCHIMIQUES DE RATS FEMELLES SOUMIS A L´ENTRAINEMENT DE NATATION.**

RÉSUMÉ

L'objectif de cette recherche a été celui d'étudier les paramètres biochimiques et nutritionel(s) de rats femelles soumis a la malnutririon protéique-calorique à la naissance et postérieur récupération nutritionelle, associée ou non à l´exercice physique (natation pendant 30 minutes par jour, 5 jours par semaine pendant 8 semaines). Ont a utilisé 32 rats Fishes divisées en quatre groupes : sédentaire, entraîné, guéri sédentaire, guéri entraîné. On a étudié le gain de poids, les apports alimentaires et les concentrations sériques des protéines totales, albumine, hémoglobine et de glucose. On a observé que l´entraînement produit l´augment de la consommation alimentaire, sans toutefois conduire à un gain de poids significatif, même en animaux en réadaptation nutritionelle. Les évaluations ont montré que les animaux ne présentent pas de récupération nutritionnelle au cours de la période étudiée et que l´entraînement n'a pas été en mesure d'intervenir dans ce procès. L'interaction entre l'état nutritionnel et entraînement autant pour l´ingestion alimentaire autant pour la concentration de sérum de glucose points à la nécessité d'étudier ce modèle avec la utilisation d'autres indicateurs du métabolisme des hydrates de carbone, par exemple, une courbe de tolérance au glucose, en plus d´autres modèles d´exercice plus intense ou à une durée plus étendue.

MOTS-CLÉS: Dénutrition, la récupération nutritionelle, l'exercice.

EFFECTOS DE LA DESNUTRICIÓN PROTEICO-CALÓRICA PRECOZ Y DE LA RECUPERACIÓN NUTRICIONAL SOBRE PARÁMETROS BIOQUÍMICOS DE RATAS HEMBRAS SOMETIDAS A ENTRENAMIENTO DE NATACIÓN.

RESUMEN

El objetivo de este trabajo fue estudiar parámetros bioquímicos y nutricionales de ratas hembras sometidas a desnutrición proteico-calórica al nacimiento y posterior recuperación nutricional asociada o no al ejercicio físico (natación 30 minutos por día, 5 días por semana durante 8 semanas). Fueron utilizadas 52 ratas Fischer hembras, distribuidas en seis grupos: Control Sedentario (CS), Control Entrenado (CE), Recuperado Sedentario (RS) y Recuperado Entrenado (RE). Hemos estudiado la ingestión alimentar, el gaño de peso y las concentraciones séricas de proteínas totales, albumina, hemoglobina y glucosa. Fue observado que el entrenamiento aplicado ha generado consumo alimentar más elevado aunque no implicando gaño de peso significativo, mismo entre los animales en recuperación nutricional. La evaluación bioquímica efectuada mostró que los animales no presentaran recuperación nutricional en el período estudiado y que el entrenamiento aplicado no fue capaz de interferir en este proceso. La interacción entre estado nutricional y entrenamiento tanto para ingestión alimentar cuanto para concentraciones séricas de glucosa apunta para la necesidad de estudiarse el presente modelo pero aplicando otros indicadores del metabolismo de carbohidratos como por ejemplo la curva de tolerancia a la glucosa y otros modelos de ejercicio más intensos o con volumen de trabajo más grande.

PALABRAS-LLAVE: Desnutrición, recuperación nutricional, ejercicio.

EFEITOS DA DESNUTRIÇÃO PROTÉICO-CALÓRICA PRECOCE E DA RECUPERAÇÃO NUTRICIONAL SOBRE PARÂMETROS BIOQUÍMICOS DE RATAS SUBMETIDAS AO TREINAMENTO DE NATAÇÃO.

RESUMO

O objetivo desse trabalho foi estudar parâmetros bioquímicos e nutricionais de ratas submetidas à desnutrição protéico-calórica ao nascimento e posterior recuperação nutricional, associada, ou não, ao exercício físico (natação 30 minutos por dia, 5 dias por semana durante 8 semanas). Foram utilizadas 32 ratas Fisher, distribuídas em quatro grupos: Controle Sedentário (CS), Controle Treinado (CT), Recuperado Sedentário (RS); Recuperado Treinado (RT). Estudamos o ganho de peso, a ingestão alimentar e as concentrações séricas de proteínas totais, albumina, hemoglobina e glicose. Foi observado que o treinamento aplicado gera um maior consumo alimentar sem, contudo levar a um ganho de peso significativo, mesmo em animais em processo de recuperação nutricional. As avaliações realizadas mostraram que os animais não apresentaram uma recuperação nutricional no período estudado e que o treinamento aplicado não foi capaz de interferir nesse processo. A interação entre estado nutricional e treinamento tanto para ingestão alimentar quanto para concentrações séricas de glicose sinaliza para necessidade de se estudar o presente modelo aplicando outros indicadores do metabolismo de carboidratos como, por exemplo, uma curva de tolerância oral à glicose, além é claro de outros modelos de exercício e mesmo exercícios mais intensos ou com um maior volume de trabalho.

PALAVRAS-CHAVE: Desnutrição, recuperação nutricional, exercício.