82 - OXIDATIVE STRESS AND LIPID PROFILE IN MALNOURISHED AND EXERCISED ANIMALS

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INTRODUCTION

It has been recently observed that children with malnutrition of the marasmus type showed lower values of paraoxonase activity and higher concentration of peroxide in the plasma than normal ones. This indicates an increase in pro-oxidant activity and a reduction in the antioxidant capacity in malnourished children. (Ece et al., 2007).

Paraoxonase (PON-1) is an enzyme produced in the liver and secreted into the blood stream linked to the high density lipoprotein (HDL) (Kelso et al., 1994). There is evidence that HDL prevents atherosclerosis by inhibiting the oxidation of low density lipoprotein (LDL). Monocyte infiltration in the artery wall needs the stimulatory effect of oxidized LDL. HDL associated to the enzymes PON-1 and lecitin cholesterol acyl transferase (LCAT) inhibit LDL oxidation (Bielicki & Forte, 1999). The antiatherogenic potential of paraoxonase comes from its capacity of hydrolizing oxidized lipids and phospholipids and thus preventing them from accumulating in the LDL particles. Besides LDL oxidation there is evidence in animals and in *in vitro* models that paraoxonase can protect HDL against its auto-oxidation thus maintaining its integrity. (Oda et al., 2002). As HDL removes the excess of cholesterol from the tissues (reverse transportation of cholesterol) and inhibits the inflammatory process the preservation of the HDL particle can be the beneficial role of paraoxonase.

Physiological factors like exercise (Tsakiris et al., 2007); pathological ones like viral and bacterial infections and diabetes (Aviram 2004) besides malnutrition (Ece et al., 2007) affect PON-1 activity. Dietetic factors are potent modulators too. Fat-rich diets reduce its activity (Thomàs-Moyà et al., 2007) while food antioxidants like polyphenols increase PON-1 expression and activity (Aviram & Rosenblat, 2005). Neverthekess PON-1 activity has not yet been determined in a study that puts together both physical exercise and diet.

Sen e Packer (2000) caracterize thiols as multifuntional molecules and amongst these function they play a role as antioxidants and are characterized by the presence of sulphydryl groups. Thus the dosage of sulphydryls can be one more indicative of the oxidative stress suffered by the organism since by measuring the concentration of antioxidants present in a given system, the degree of oxidative stress can be estimated.

OBJECTIVE

To evaluate the effect of physical training of low volume and intensity on the oxidative stress and lipid profile of female rats submitted to post-weaning protein-calorie malnutrition.

MATERIAL AND METHODS

Animals

Twenty eight female Fisher rats were divided into four groups: control sedentary (CS), control trained (CT), 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 hole 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. Thus it was possible to calculate

Food Efficiency (weight gain/food ingestion). 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. Determination of total and free sulphydryls was performed as described by Sedlak & Lindsay (1968). Bound sulphydryls was obtained by subtracting free from total ones. Paraoxonase activity was determined as described by Beltowski et al. (2002).

Statistical analysis

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

RESULTS

NUTRITIONAL AND BIOCHEMICAL PARAMETERS

Body weight and food efficiency were statistically higher in the control animals than in the malnourished ones (Table II) and the same happened with CK and PON activities (Table III).

Table II - Body weight and food efficiency (FE) of animals of groups Sedentary Control (SC), Trained Control (TC), Sedentary Malnourished (SM) and Trained Malnourished (TM) after nine weeks of experiment.

Groups\Parameters	Body weight (g)	FE
SC	207.17 ± 13.25[a]	0.09 ± 0.04
TC	201.17 ± 13.18[a]	0.08 ± 0.02
SM	53.14 ± 14.99[c]	0.03 ± 0,05
TM	74.57 ± 19.20[b]	0.05 ± 0.07
P value (Anova Two Way)		
Nutritional status	P<0.0001	0.0012
Training	NS	NS
Interaction	0.0216	NS

Results are expressed as mean ± standard deviation. NS = non significant. Different letters indicate significant difference $p < 0.05$. Food efficiency = weight gain/ food ingestion.

Table III - Creatine Kinase (CK) and paraoxonase (PON) activity of animals of groups Sedentary Control (SC), Trained Control (TC), Sedentary Malnourished (SM) and Trained Malnourished (TM) after nine weeks of experiment.

Groups\Parameters	CK (U/L)	PON (U/L)
SC	1.53 ± 0.44	252.16 ± 17.82
TC	1.74 ± 0.82	246.45 ± 8.31
SM	0.54 ± 0.43	53.12 ± 6.68
TM	0.60 ± 0.73	57.07 ± 10.26
P value (Anova Two Way)		
Nutritional status	0.0001	P<0.0001
Training	NS	NS
Interaction	NS	NS

Results are expressed as mean ± standard deviation. NS = non significant. Different letters indicate significant difference $p < 0.05$. Dosage of CK performed using laboratory kit.

On the other hand total and HDL cholesterol concentrations were statistically higher in the malnourished group as compared with controls (Table IV).**Table IV** - Concentration of Total, HDL and other fractions (VLDL and LDL) cholesterol of animals of groups Sedentary Control (SC), Trained Control (TC), Sedentary Malnourished (SM) and Trained Malnourished (TM) after nine weeks of experiment.

Groups\Parameters	Total Cholesterol (mmol/L)	HDL (mmol/L)	VLDL e LDL (mmol/L)
SC	1.88 ± 0.30	1.15 ± 0.23	0.73 ± 0.17
TC	1.66 ± 0.17	1.13 ± 0.09	0.54 ± 0.12
SM	2.28 ± 0.39	1.58 ± 0.16	0.70 ± 0.24
TM	2.25 ± 0.72	1.37 ± 0.20	0.88 ± 0.73
P value (Anova Two Way)			
Nutritional Status	0.0053	P<0.0001	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, free and bound sulphydryls also showed higher values amongst the malnourished rats than in the control group (Table V).

Table V - Concentration of total, free and bound sulphydrys of animals of groups Sedentary Control (SC), Trained Control (TC), Sedentary Malnourished (SM) and Trained Malnourished (TM) after nine weeks of experiment.

Groups\Parameters	Total Sulphydryls (imol/L)	Free Sulphydryls (imol/L)	Bound Sulphydryls (i mol/L)
SC	185.43 ± 118.57	94.05 ± 46.04[b]	85.98 ± 176.63
TC	209.74 ± 90.27	164.37 ± 28.33[a]	45.36 ± 82.37
SM	427.05 ± 19.75	153.17 ± 18.79[a]	273.88 ± 33.93
TM	486.51 ± 125.96	164.89 ± 32.83[a]	321.62 ± 133.71
P value (Anova Two Way)			
Nutritional Status	P<0.0001	0.0332	P<0.0001
Training	NS	0.0045	NS
Interaction	NS	0.0362	NS

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

Interaction between nutritional status and physical training

This interaction was evidenced in the body weight of the rats; statistically higher values were encountered in SC and TC groups which were similar to each other but higher than TM. SM animals had the lowest values (Table II).

Free sulphydryls of animals in groups SM, TM and SC were statistically higher than those in group SC (Table IV).

Discussion

The higher body weight of the control animals at the end of the experiment is a reflex of their food efficiency and this fact confirms the applicability of our malnutrition protocol (Oliveira et al., 2007). Although there was no significant difference for body weight as an effect of training, mainly when the equality between SC and TC is considered, an interaction was observed between nutrition and training which was generated especially by the values shown by SM and TM animals. Thus physical exercise was beneficial to the TM animals, which had values higher than SM.

In the search for a marker of possible muscle damage induced by the training protocol the determination of CK was introduced in this experiment. We observed that a slight increase in the activity of this enzyme in the trained rats was not statistically significant and this justifies the utilization of the protocol even in the malnourished ones.

We observed that malnutrition promoted a significant increase in the concentrations of total and HDL cholesterol and that exercise was not capable of reversing this situation. As paraoxonase is linked to the HDL molecule we expected the activity of the enzyme to be also augmented. Nevertheless what we observed was exactly the opposite: a significant decrease in PON activity in the malnourished animals. Results in animal models demonstrate the antiatherogenic protection of PON. PON deficient mice are not able to protect their LDL of oxidation (Shih et al., 1998) while transgenic mice with human HDL (which had two to three times increased plasma PON activity) were more protected against LDL oxidation and in dose-dependent manner (Tward et al., 2002). Considering that PON is present in HDL we may conclude that HDL of the malnourished animals need to be studied and if our hypothesis is confirmed HDL of malnourished animals could not perform its expected antioxidant role.

The significant increase in total, free and bound sulphydryls in the malnourished animals makes us believe that malnutrition promotes a rise in free radicals formation. On the other hand the significant difference in the concentration of free sulphydryls as a function of training occurred mainly due to the increase generated by exercise in TC animals once there was no difference between SM and TM ones.

According to Hum et al. (1992) the concentrations of glutathione, methionine and cystein in the plasma increase with the increase of diet proteins, that is, malnutrition provokes the decrease of these parameters. Nevertheless they also found the glutathione concentration in live to be an indirect biomarker better than its plasmatic concentration although not better than the plasmatic cystein concentration. Our results of augmented sulphydryls in the malnourished animals can only be explained by the above set of data, since a decrease should be expected. On the other hand the correlation between diet protein concentration and that of oxidative stress markers is not good. Furthermore there may be other factors in malnutrition that could rise the sulphydryls concentration.

In relation to the effect of exercise on the concentration of free sulphydryls data indicate exercise to have been beneficial to the exercised animals due to the increase of antioxidant defenses linked to the free sulphydryls, such as increased cystein, glutathione, homocystein and others. We consider to be important the determination of these parameters in order to confirm this hypothesis.

CONCLUSION

The swimming physical training applied did not cause muscle damage detectable by the biochemical marker creatine kinase although causing positive modifications in the body weight of the malnourished trained animals. Malnutrition promoted a significant increase in total and HDL cholesterol and exercise was not able to reverse this condition. The rise in the concentration of HDL in the malnourished rats was not followed by an increase in the paraoxonase activity and this allows the formulation of the hypothesis that in malnourished animals HDL is not performing its expected antioxidant role. The rise in total, free and bound sulphydryls in malnourished animals needs to be studied together with other markers of oxidative stress.

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OXIDATIVE STRESS AND LIPID PROFILE IN MALNOURISHED AND EXERCISED ANIMALS

ABSTRACT

The goal of the present work was to study the oxidative stress and lipid profile in malnourished animals submitted to physical exercise (swimming 30 minutes/day, 5 days/week during 8 weeks). Twenty eight female Fisher rats were divided into four groups: Sedentary Control (SC), Trained Control (TC), Sedentary Malnourished (SM) and Trained Malnourished (TM). We have concluded that: a) the applied swimming physical training did not cause muscle damage detectable by the biochemical marker creatine kinase, although it caused positive modifications in the body weight of the TM animals; b) malnutrition promoted a significant increase in the concentrations of total and HDL cholesterol but physical exercise was not capable of reverting this situation; c) The rise of HDL concentration in malnourished animals were not followed by increase in the paraoxonase activity and this allows us to formulate the hypothesis that HDL of these animals might not act as antioxidants as expected for these molecules; d) the increase in total, free and bound sulphydrils in malnourished animals needs to be studied together with other markers of oxidative stress.

KEY WORDS: Malnutrition, exercise, oxidative stress.

STRESS OXIDATIF ET PROFIL LIPIDIQUE DES ANIMAUX SOUS-ALIMENTÉS ET ENTRAÎNÉS

RÉSUMÉ

L'objectif de cette recherche a été celui d'étudier le stress oxidatif et le profil lipidique des animaux sous-alimentés et soumis à la pratique d' exercices physiques (natation 30 minutes chaque jour, 5 jours par semaine pendant 8 semaines). On a fait l'expérience avec 28 rats Fisher, separées dans 4 groups: Contrôle Sédentaire (CS), Contrôle Traîné (CT), Sous-Alimenté Sédentaire (SS) e Sous-Alimenté Traîné (ST). On a conclu que: a) l'entraînement physique da la natation n'a pas porté des péjudices musculaires visibles selon le tableau biochimique creatine quinase, bien qu'il a porté des changements positifs sur le poids corporel des animaux ST; b) la sous-alimentation a augmenté considérablement les concentrations du cholésterol total et HDL (l'exercice physique n'a pu changer ce quadre); c) les élévations des concentrations de HDL chez les animaux sous-alimentés n'ont pas été suivis d'élévation de la paraoxonase, ce que permet la formulation de l'hypothèse selon laquelle les HDL des animaux sous-alimentés peuvent n'accomplir pas la fonction antioxidante prévisible pous cettes molécules; d) l'augmentation dans les concentrations des sulfidriles totales, libérées et liées aux animaux sous-alimentés, a besoin d'être étudiée en ensemble avec d'autres tableaux de stress oxidatif.

MOTS-CLÉS: exercices physiques, sous-slimenté, stress oxidatif.

STRESS OXIDATIVO Y EL PERFIL LIPÍDICO DE ANIMALES MALNUTRIDOS Y EJERCIDOS

RESUMEN

El objetivo de este trabajo fue estudiar el stress oxidativo y el perfil lipídico de animales desnutridos y sometidos al ejercicio físico (natación 30 minutos por día, cinco dias por semana durante 8 semanas). Fueron utilizadas 28 ratas Fisher hembras distribuidas en cuatro grupos: Controle Sedentario (CS), Controle Entrenado (CE), Desnutrido sedentario (DS) y Desnutrido Entrenado (DE). Hemos concluido que: a) el entrenamiento físico de natación no ha causado daños musculares detectables por el marcador bioquímico creatina quinasa, a pesar de causar modificaciones positivas en el peso corporal de los animales DE; B) la desnutrición ha promovido un significativo aumento en las concentraciones de colesterol total y HDL pero el ejercicio no fue capaz de reverter este cuadro; C) los aumentos en las concentraciones de HDL en los animales desnutridos no han sido seguidos de aumento de la actividad de la paraoxonasa , lo que abre camino para formulación de la hipótesis de que las HDL's de los animales desnutridos pueden no desempeñar la función antioxidante esperada para esas moléculas; d) El aumento en las concentraciones de las sulfidrilas totales, libres y ligadas precisan ser estudiadas junto con otros marcadores del stress oxidativo.

PALABRAS-LLAVE: Desnutrición, ejercicio, stress oxidativo.

ESTRESSE OXIDATIVO E PERFIL LIPÍDICO DE ANIMAIS DESNUTRIDOS E EXERCITADOS

RESUMO

O objetivo desse trabalho foi estudar o estresse oxidativo e o perfil lipídico de animais desnutridos e submetidos ao exercício físico (natação 30 minutos por dia, 5 dias por semana durante 8 semanas). Foram utilizadas 28 ratas Fisher, distribuídas em quatro grupos: Controle Sedentário (CS), Controle Treinado (CT), Desnutrido Sedentário (DS) e Desnutrido Treinado (DT). Concluímos que: a) o treinamento físico de natação aplicado não causou danos musculares detectáveis pelo marcador bioquímico creatina quinase, apesar de causar modificações positivas ocorridas no peso corporal dos animais DT; b) a desnutrição promoveu um aumento significativo nas concentrações de colesterol total e HDL, e o exercício não foi capaz de reverter esse quadro; c) os aumentos nas concentrações de HDL nos animais desnutridos não foram seguidos de aumento na atividade da paraoxonase, o que abre caminho para formulação da hipótese de que as HDLs dos animais desnutridos podem não desempenhar a função antioxidante esperada para essas moléculas; d) o aumento nas concentrações das sulfidrilas totais, livres e ligadas em animais desnutridos precisam ser estudadas conjuntamente com outros marcadores de estresse oxidadativo. PALAVRAS-CHAVE: Desnutrição, exercício, estresse oxidativo.